CARBON SORBENT REINFORCED HOLLOW FIBER MEMBRANE MICROEXTRACTION FOR DETERMINATION OF TRACE POLAR ORGANIC COMPOUNDS

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การสกัดระดับจุลภาคด้วยเมมเบรนเส้นใยกลวงที่เสริมแรงด้วยตัวดูดซับคาร์บอนสำหรับการตรวจวัดสารประกอบอินทรีย์มีขั้วปริมาณน้อย

น.ส.ชนัดดา วรวิชญ์

วิทยานิพนธ์นี้เป็นส่วนหนึ่งของการศึกษาตามหลักสูตรปริญญาวิทยาศาสตรดุษฎีบัณฑิต
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Thesis Title	CARBON SORBENT REINFORCED HOLLOW FIBER MEMBRANE MICROEXTRACTION FOR DETERMINATION OF TRACE POLAR ORGANIC COMPOUNDS
By	Miss Chanatda Worawit
Field of Study	Chemistry
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ชนัดดา วรวิชญ์ : การสกัดระดับจุลภาคด้วยเมมเบรนเส้นใยกลวงที่เสริมแรงด้วยตัวดูดซับคาร์บอนสำหรับการตรวจวัดสารประกอบอินทรีย์มีขั้วปริมาณน้อย . (CARBON SORBENT REINFORCED HOLLOW FIBER MEMBRANE MICROEXTRACTION FOR DETERMINATION OF TRACE POLAR ORGANIC COMPOUNDS) อ.ที่ปรึกษาหลัก : รศ. ดร.ปกรณ์ วรานุศุภากุล

งานวิจัยนี้นำเสนอแนวความคิดในการสกัดระดับจุลภาคด้วยเมมเบรนเส้นใยกลวงที่เสริมแรงด้วยวัสดุคาร์บอนระดับนาโน เพื่อเพิ่มประสิทธิภาพการสกัดสารประกอบอินทรีย์มีขั้วปริมาณน้อย โดยศึกษาตัวดูดซับคาร์บอนหลากหลายชนิด อาทิ กราไฟต์ กราฟีน คาร์บอนนาโนไฟเบอร์ ในงานวิจัยที่หนึ่ง เป็นการพัฒนาการสกัดระดับจุลภาคด้วยเมมเบรนเส้นใยกลวงที่เสริมแรงด้วย กราไฟต์สำหรับตรวจวัดสารประกอบไตรฮาโลมีเทนในตัวอย่างน้ำ มีการศึกษาและหาสภาวะที่เหมาะสมซึ่งเป็นปัจจัยที่มีผลต่อประสิทธิภาพการสกัด ทั้งชนิดของตัวทำละลายอินทรีย์ที่ใช้เป็นตัวพยุงเมมเบรนเส้นใยกลวง ปริมาณกราไฟต์ที่ใส่ รวมทั้งระยะเวลาในการสกัด ได้ช่วงความเป็นเส้นตรงในช่วงความเข้มข้น 0.2 ถึง 120 ไมโครกรัมต่อลิตร (อาร์สแคว > 0.99) ใช้ระยะเวลาการสกัด 10 นาที ค่าความเข้มข้นต่ำสุดที่ตรวจวัดอยู่ในช่วงความเข้มข้น 0.01 ถึง 0.1 ไมโครกรัมต่อลิตร ค่าเอนริชเมนท์แฟคเตอร์ที่บ่งบอกถึงความสามารถในการเพิ่มความเข้มข้นของวิธีอยู่ในช่วง 40 ถึง 71 เมื่อเปรียบเทียบความไวในการวิเคราะห์ของวิธีนี้กับวิธีการสกัดระดับจุลภาคด้วยเมมเบรนเส้นใยกลวงทั่วไป พบว่างานวิจัยนี้ใช้ระยะเวลาในการสกัดที่น้อยกว่า นั่นคือการเพิ่มกราไฟต์ในวิธีการสกัดระดับจุลภาคด้วยเมมเบรนเส้นใยกลวงสามารถเพิ่มประสิทธิภาพการสกัดได้ นอกจากนี้ยังมีการพัฒนาการสกัดระดับจุลภาคด้วยเมมเบรนเส้นใยกลวงที่เสริมแรงด้วยตัวดูดซับคาร์บอนให้สามารถต่อกับเครื่องโครมาโทกราฟีของเหลวสมรรถนะสูงสำหรับการวิเคราะห์แบบอัตโนมัติ โดยงานวิจัยที่สอง เป็นการพัฒนาเทคนิคการสกัดระดับจุลภาคด้วยเมมเบรนเส้นใยกลวงที่เสริมแรงด้วยคาร์บอนนาโนไฟเบอร์ให้สามารถสกัดและตรวจวัดยาที่มีคุณสมบัติเป็นกรดในกลุ่มยาต้านการอักเสบชนิดไม่มีสเตียรอยด์ในตัวอย่างปัสสาวะ อุปกรณ์ที่ใช้สำหรับการสกัดระดับจุลภาคด้วยเมมเบรนเส้นใยกลวงออกแบบและสร้างด้วยเครื่อง 3D พริ้นเตอร์และต่อเข้ากับเครื่องโครมาโทกราฟีของเหลวสมรรถนะสูงเพื่อใช้ในการสกัดและหาปริมาณสาร งานวิจัยนี้มีการศึกษาและหาสภาวะที่เหมาะสมซึ่งเป็นปัจจัยที่มีผลต่อประสิทธิภาพการสกัด ทั้งชนิดของตัวทำละลายอินทรีย์ที่ใช้เป็นตัวพยุงเมมเบรนเส้นใยกลวง สภาวะของสารละลายให้และรับ รวมทั้งกระบวนการในการสกัดแบบอัตโนมัติที่ต่อเข้ากับเครื่องโครมาโทกราฟีของเหลวสมรรถนะสูง พบว่าระยะเวลาในการสกัดสาร 1 ตัวอย่างใช้เวลา 47 นาที และสามารถวิเคราะห์สารตัวอย่าง 4 ตัวอย่างใน 1 ชั่วโมง ค่าความเข้มข้นต่ำสุดที่ตรวจวัดอยู่ในช่วงความเข้มข้น 1.6 ถึง 5.6 ไมโครกรัมต่อลิตร ร้อยละการคืนกลับของยาต้านการอักเสบชนิดไม่มีสเตียรอยด์ในตัวอย่างปัสสาวะ อยู่ในช่วงร้อยละ 97 ถึง 105 ร้อยละการเบี่ยงเบนมาตรฐานสัมพัทธ์อยู่ในช่วงร้อยละ 0.3 ถึง 4.6 ดังนั้นการสกัดระดับจุลภาคด้วยเมมเบรนเส้นใยกลวงที่เสริมแรงด้วยตัวดูดซับคาร์บอนสามารถเพิ่มประสิทธิภาพการสกัด ส่งผลให้ค่าเอนริชเมนท์แฟคเตอร์สูงขึ้นและลดระยะเวลาในการวิเคราะห์ลง ถือเป็นข้อดีของการสกัดแบบอัตโนมัติที่จะได้จำนวนการวิเคราะห์สารตัวอย่างมากขึ้น

สาขาวิชา เคมี ลายมือชื่อนิสิต .....

ปีการศึกษา 2561 ลายมือชื่อ อ.ที่ปรึกษาหลัก .....

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KEYWORD: Automatic / Carbon nanomaterials / Hollow fiber liquid phase microextraction / Acidic drugs / Trihalomethanes

Chanatda Worawit : CARBON SORBENT REINFORCED HOLLOW FIBER MEMBRANE MICROEXTRACTION FOR DETERMINATION OF TRACE POLAR ORGANIC COMPOUNDS. Advisor: Assoc. Prof. PAKORN VARANUSUPAKUL, Ph.D.

This work presents a proof of concept that hollow fiber liquid phase microextraction reinforced with carbon nanomaterials would improve the extraction efficiency of small and relatively polar organic compounds. Several types of carbon sorbents such as graphite, graphene, carbon nanofiber were studied. In research I, the hollow fiber membrane reinforced with graphite was developed for liquid phase microextraction of trihalomethanes in water samples. Parameters affecting extraction efficiency including organic solvent as supported liquid membrane, amount of loaded graphite and extraction time were investigated and optimized. The working range of 0.2-120 μg L^{-1} was obtained in 10 min extraction with good linearity (R^2 > 0.99). Limit of detections were in the range of 0.01-0.1 μg L^{-1} with enrichment factors of 40-71. The method offered comparative sensitivity to the conventional HF-LPME with significantly shortened extraction time indicating that the addition of graphite to the HF-LPME could improve the extraction efficiency. In addition, the carbon sorbent reinforced HF-LPME was developed and configured for in-line extraction coupled to a chromatographic system for automated analysis. In research II, carbon nanofiber reinforced HF-LPME was developed and configured for simultaneous in-line extraction and detection of nonsteroidal anti-inflammatory acidic drugs in urine samples. The hollow fiber extraction chamber was designed and fabricated by a 3D printer and connected to high performance liquid chromatographic system (HPLC) for simultaneous in-line extraction and determination. Parameters affecting extraction efficiency including organic solvent as supported liquid membrane, donor and acceptor condition, and analytical sequence of automatic extraction and HPLC determination were investigated and optimized. The analytical sequence of automatic extraction and HPLC determination provided total analysis time of 47 min and the sample throughput of 4 samples hr^{-1} was achieved. The limit of detections for the method were in the range from 1.6-5.6 μg L^{-1}. The recoveries in real human urine samples were in the range of 97-105 % and relative standard deviations were between 0.3-4.6 %. The carbon sorbent reinforced HF-LPME can enhance the extraction efficiency resulting in higher enrichment factor and shorter extraction time, which offer an advantage towards in-line extraction and automation to achieve high sample throughput.

Field of Study:	Chemistry	Student's Signature .....
Academic Year:	2018	Advisor's Signature .....

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Chanatda Worawit

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LIST OF ABBREVIATIONS

%	percentage
^{0}C	degree Celsius
cm	centimeter
CNFs	carbon nanofibers
cP	centipoise
DI-SDME	direct immersion single-drop microextraction
DIC	diclofenac
DLLME	dispersive liquid liquid microextraction
DWCNTs	double-walled nanotubes
ECD	electron capture detector
EE	extraction efficiency
EF	enrichment factor
EME	electromembrane extraction
g	gram
GC	gas chromatography
HPLC	high performance liquid chromatography
HS-SDME	headspace single-drop microextraction
IBU	ibuprofen
MWCNTs	multi-walled nanotubes
SWCNTs	single-walled nanotubes
I.D.	internal diameter
KET	ketoprofen
LLE	liquid-liquid extraction
LODs	limit of detections
LOQs	limit of quantifications
HF-LPME	hollow-fiber liquid-phase microextraction
LPME	liquid-phase microextraction
M	molar

$mg \cdot L^{-1}$	milligram per liter
min	minute
mL	milliliter
mM	millimolar
mm	millimeter
NAP	naproxen
$ng \cdot mL^{-1}$	nanogram per milliliter
NSAIDs	non-steroidal anti-inflammatory drugs
R	recovery
R^2	correlation coefficient
rpm	revolutions per minute
R.S.D.	relative standard deviation
SEM	scanning electron microscope
SD	standard deviation
SDME	single-drop microextraction
SLM	supported liquid membrane
SPE	solid-phase extraction
SPME	solid-phase microextraction
S/N	signal to noise ratio
THMs	trihalomethanes
USEPA	The United States Environmental Protection Agency
UV-Vis	ultraviolet-visible
WHO	World Health Organization
$\mu g \cdot L^{-1}$	microgram per liter
µL	microliter
µm	micrometer

CHAPTER I
INTRODUCTION

1.1 Motivation of proposer

The determination of trace organic species in various samples is of a great interest. Direct determination is sometimes difficult due to low concentration of analytes and matrix interferences in samples. For these reasons, sample preparation is a key step in an analytical process. There are two purposes for sample preparation process; isolation of target analytes from complicated sample matrices that may interfere with the detection system and preconcentration of trace analytes having final concentration sufficient for the instrument detection limit. The most common method in sample preparation technique is liquid-liquid extraction (LLE) but it consumes large amount of sample and organic solvent, and it is time-consuming and harmful to environment and human [1]. Later, liquid phase microextraction (LPME) has been developed to overcome these drawbacks.

There are several LPME techniques have been reported. One is dispersive liquid liquid microextraction (DLLME), where the target analytes are extracted from aqueous samples into a dispersed extracting. Single drop microextraction (SDME), where the target analytes are extracted from aqueous samples into a microliter drop of the organic solvent hanging at the needle tip of a microsyringe. However, both techniques have some limitations such as difficult to collect the droplet after extraction and unable to control the droplet size. In order to overcome this problem, hollow-fiber membrane liquid phase microextraction (HF-LPME) has been developed.

Hollow-fiber liquid phase micro extraction (HF-LPME) is a simple, fast and environmental friendly method for effective extraction and preconcentration of trace analytes from various kinds of samples [2]. The extraction process involves mass transfer of the analyte mainly based on passive diffusion of the analyte from the donor solution to the acceptor solution. HF-LPME consumes such a small volume of organic solvent so that it provides high enrichment factor. Since the extraction by HF-LPME is

based on passive diffusion and distribution ratio of the analyte and the organic solvent. HF-LPME is effective for extraction of relatively non-polar organic compounds having relatively high distribution constant (K) whereas HF-LPME of relatively polar organic compounds with relatively low distribution constant (K) is possible; however, it may have taken a long extraction time to achieve the sufficient concentration for analysis. Recently, sorbent reinforced HF-LPME such as carbon nanomaterials has been introduced to provide additional extraction mechanism due to its large specific surface area and its ability to establish intermolecular interaction.

Carbon-nanomaterials are held in the lumen of the HF or immobilized into the pores of the HF and serve as a trap, providing a rapid solute transport and improving the extraction efficiency [8]. Most applications are for extraction of relatively large and non polar organic compounds such as aromatic compounds, pesticides, drugs, flavonoids from various sample matrices; for example, human blood, urine, breast milk, serum, beverage, ecological textiles eggs and pork [3-7]. So, the author is interested in developing the method for extraction and preconcentration of small and relatively polar organic compounds (low K). In this work, trihalomethanes, which are small and relatively polar organic compounds will be used as model analytes.

In addition, despite several developments in HF-LPME have been reported over the past few years, only off-line extraction and semi-automatic microfluidic setups have been developed [8-13]. Another interesting issue is to develop in-line HF-LPME and couple to a chromatographic separation system for simultaneous extraction and determination of mix target analytes in real samples. In this work, non-steroidal anti-inflammatory drugs (NSAIDs) were selected as model analytes, which can be detected by high performance liquid chromatography (HPLC) method.

1.2 Literature reviews

Many applications using carbon nanomaterials as sorbents or reinforced liquid phase microextraction have been reported.

In 2008, Xu, L. and Lee H-K. [14] used graphite fiber as a sorbent material in micro-solid phase extraction (μ-SPE) devices for extraction of polycyclic aromatic hydrocarbons (PAHs) in soil samples by microwave-assisted extraction (MAE) technique and detected by gas chromatography. The results were compared to sonication-assisted extraction (SAE) and agitation-assisted extraction (AAE). The μ-SPE devices were also compared between using normal granular activated carbon material and graphite fiber. The results showed that when using graphite fiber as sorbent, MAE provided higher chromatographic signals than the other three methods and using graphite fiber gave higher extraction efficiency than the granular activated carbon. It indicated that graphite fiber is a very good and effective material for extraction of PAHs.

In 2012, Song, X.-Y. et al. [15] studied carbon nanotubes (CNTs) reinforced HF-LPME for preconcentration of piroxicam and diclofenac in different water samples and detected by HPLC. The sample solution was extracted within 45 min. The results showed that functionalized multi-walled carbon nanotubes immobilized hollow fiber membrane has provided high enrichment factors (47- and 184-fold for piroxicam and diclofenac, respectively) and the limits of detections were 4.58 μg L^{-1} for piroxicam and 0.40 μg L^{-1} for diclofenac. The method showed high reproducibility and absence of sample carryover.

In 2013, Song, X.-Y. et al. [16] used carbon nanotubes (CNTs) reinforced hollow fiber solid-phase microextraction (HF-SPME) for determination of five carbamate pesticides in apples detected by HPLC. The sample solution was extracted within 60 min. The results showed that the CNTs reinforced HF-SPME provided high enrichment factors, good precision, simplicity, absence of carry-over.

In the same year, Hasheminasab K.S. et al. [17] developed a new design of low voltage electromembrane extraction (EME) using CNTs reinforced hollow fiber membranes for determination of acidic drugs; ibuprofen and naproxen in biological and waste water samples detected by capillary electrophoresis (CE). The results showed that the recovery increased when increasing extraction time and then constant after 10 min due to equilibrium. The finding indicated that the use of CNTs in hollow fiber membranes increases the overall analyte partition coefficient in the membrane and lead to enhancement in extraction efficiency. The EME setup was shown in Figure 1.1.

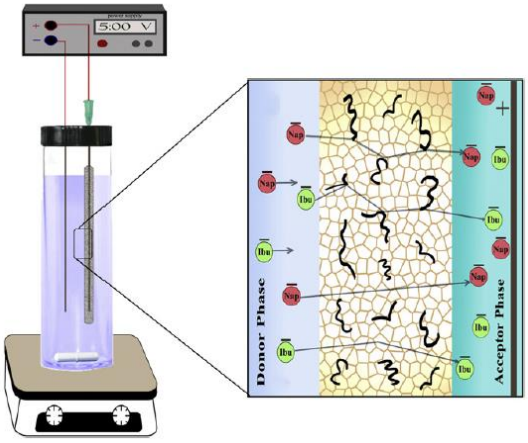

Figure 1.1 The set-up for electro membrane extraction (EME) in the presence of carbon nanotubes in SLM [17]

In 2014, Ma, X. et al. [18] used graphene reinforced HF-LPME for extraction of four carbamate residues in apples and pears followed by HPLC. The results indicated that the graphene-HF-LPME provided a low extraction time for just only 5 minutes, low detection limit in the range from 0.2 to 1.0 ng g^{-1} and good linearity in the range of 1.0-100.0 ng g^{-1}. The graphene-HF-LPME was simple and cost-effective technique.

In 2018 , Han, X.-F. et al. [19], developed N-doped carbon nanotubes-reinforced HF-LME method for determination of two naphthalene-derived phytohormones, 1-naphthalene acetic acid (NAA) and 2-naphthoxyacetic acid (2-NOA), at trace levels in tomatoes coupled with HPLC. The results showed that N-doped CNTs-HF-LPME provides effective extraction performance for NAA and 2-NOA. This method showed 165- and 123-fold enrichment factors of NAA and 2-NOA, good repeatability and reproducibility, low limits of detection and quantification (at ng g^{-1} levels). The recoveries in the range of 83-108% were reported.

In the same year, Rezazadeh, T. et al. [20] developed graphene oxide-polyaniline (GO/PANI) for preconcentration of Ivermectin in some environmental samples. The results indicated that GO/PANI had a higher adsorption efficiency for the Ivermectin in comparison with GO and GO-ethylen diamine (GO/EDA). The method showed good linear dynamic range at 0.1–5000 μg L^{-1}, limit of detection at 0.03 μg L^{-1} and excellent preconcentration factor of 220.

In 2013, Nitiyanontakit, S. et al. [21] developed the hybrid flow analyzer for automatic hollow fiber assisted ionic liquid based liquid phase microextraction with in-line membrane regeneration. This method was explored by using Cr(VI) as a model analyte, 10% (v/v) methyltrioctyl ammonium chloride in kerosene as the supported liquid membrane (SLM) and 1,5-diphenylcarbazide as the acceptor solution. The extraction time of 4.5 min were achieved with an enrichment factor of 11 and the limit of detection of 4.6 μg·L^{-1}.

As shown above, many researches have attempted to use carbon nanomaterials to improve the extraction efficiency of HF-LPME. Most applications were for determination of non-polar organic compounds or acidic and basic drugs from various sample matrices. Besides, there was an attempt to design and configure the HF-LPME for on-line extraction and automated determination system.

1.3 Objective and scope of this research

In this work, several types of carbon sorbents such as graphite, graphene, carbon nanofiber reinforced into HF-LPME were studied for extraction and determination of small and relatively polar organic compounds (low K). Trihalomethanes and acidic drugs (Nonsteroidal anti-inflammatory drug) were selected as model analytes. Parameters affecting extraction efficiency including organic solvent as supported liquid membrane, donor and acceptor condition and extraction time were investigated and optimized. In addition, the carbon reinforced HF-LPME was developed for in-line extraction and automatic analysis. Finally, applications of the methods to real samples were demonstrated.

CHAPTER II

THEORY

2.1 Liquid-phase microextraction (LPME)

Liquid phase microextraction (LPME) uses small amounts of sample solution and organic solvent to provide higher enrichment factor in the final extract than LLE. The advantages of LPME are rapid, simple, inexpensive, environmental friendly and easy to set for automation [22].

Liquid phase microextraction is non-exhaustive extraction. The analytes in the sample solution are transferred into the extracting phase via passive diffusion. It is based on equilibrium process as illustrated below:

$$A_{aq} \rightleftharpoons A_{org} \qquad \text{Equation 2.1}$$

where A_{aq} is the analyte in aqueous phase and A_{org} is the analyte in organic phase.

The distribution constant or distribution ratio ($K_{org/aq}$) is the ratio of the concentration of A in organic phase at equilibrium ($C_{eq/org}$) and the concentration of A in aqueous phase at equilibrium ($C_{eq/aq}$) as shown in Equation 2.2.

$$K_{org/aq} = \frac{C_{eq,org}}{C_{eq,aq}} \qquad \text{Equation 2.2}$$

After extraction, the extraction efficiency (EE) of the target analyte can be calculated by Equation 2.3.

$$EE = \frac{n_{org}}{C_i V_{aq}} \times 100 = \frac{K_{org/aq} V_{org}}{K_{org/aq} V_{org} + V_{aq}} \times 100 \qquad \text{Equation 2.3}$$

where n_{org} is the amount of target analyte extracted into the organic phase

C_i is the initial concentration of the target analyte in the aqueous sample

V_{aq} is the volume of sample phase

V_{org} is the volume of organic phase

The preconcentration capability of LPME technique can be expressed as enrichment factor (EF), which can be calculated by Equation 2.4.

$$EF = \frac{C_{org}}{C_i} = \frac{V_{aq} EE}{100\ V_{org}} \qquad \text{Equation 2.4}$$

where C_{org} is the concentration of the target analyte in the organic phase after extraction process [23].

The enrichment factor (EF) is inversely proportional to the volume of organic solvent (V_{org}) and directly proportional to the extraction efficiency (EE). Typically, the number of the volume ratio (V_{aq}/V_{org}) can be varied in tens to hundreds while the extraction efficiency (EE) of this technique is quite low, which varied between 0-1 depending to the distribution constant ($K_{org/aq}$). Nevertheless, if the volume ratio is kept constant, the enrichment factor can be improved by increased extraction efficiency as a result of increased distribution ratio.

Liquid phase microextraction is available in many configurations.

2.1.1 Dispersive liquid liquid microextraction (DLLME)

Dispersive liquid liquid microextraction (DLLME) is based on ternary component solvent system. In this method, the mixture of extracting and dispersing solvents is injected into aqueous sample rapidly by a syringe. Thereby, cloudy solution is formed. After centrifugation, the fine drops of extraction solvent are sedimented in the bottom of the conical centrifuge tube. Finally, the extracting solvent containing analytes is

collected from the bottom of the centrifuge tube for analysis with an analytical instrument [24]. The DLLME procedure is shown in Figure 2.1. The advantages of DLLME method are simplicity of operation, rapidity, low cost, high recovery, and high enrichment factor but the choices of extracting solvent are limited and the collection of extracting phase is sometimes troublesome.

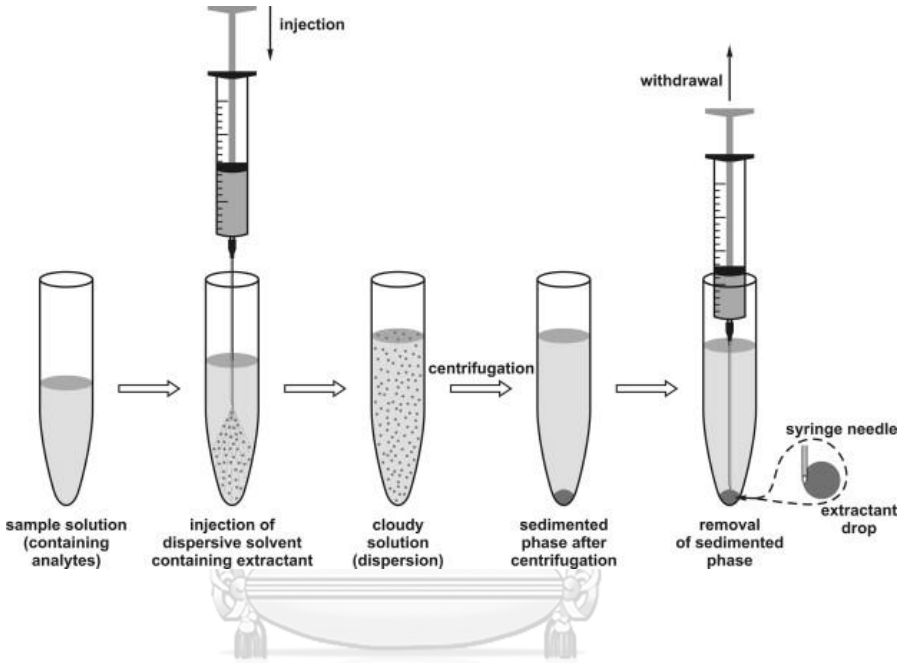

Figure 2.1 Procedure of dispersive liquid-liquid microextraction [25]

2.1.2 Single drop microextraction (SDME)

Single drop microextraction (SDME) is one of the LPME configuration. The target analytes are extracted from aqueous samples into a microliter drop of the organic solvent hanging at the needle tip of a microsyringe [26]. SDME can be operated into two main modes; direct immersion (DI)-SDME and headspace (HS)-SDME. In direct immersion SDME, a small drop of an organic solvent is suspended at the tip of the microsyringe needle that is immersed in a stirred aqueous sample solution as shown in Figure 2.2 a). The target analytes are extracted into the organic hanging droplet. After extraction, the droplet of organic solvent is withdrawn into the microsyringe and then directly injected into the analytical instrument. This SDME mode is suitable for

extraction of medium polarity and non-polar semi and non-volatile analytes. In headspace SDME, the organic solvent drop is suspended in the headspace of the heated sample solution as shown in Figure 2.2 b). This configuration is suitable for extraction of volatile and semi-volatile analytes. SDME provides a simple, inexpensive, and environmental friendly technique, but there are some limitations, for examples; the solvents are limited to low vapor pressure to avoid evaporation during sampling, the solvent should be compatible with GC analysis, and it is difficult to operate and control the drop size. Hence, hollow fiber liquid phase microextraction (HF-LPME) was developed to overcome these drawbacks [22].

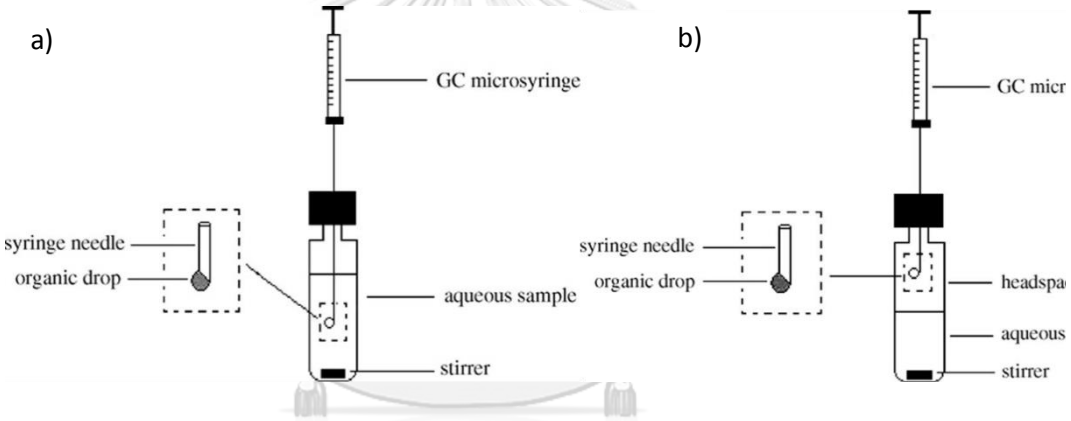

Figure 2.2 Setups of single drop microextraction (SDME); a) (DI)-SDME mode; and b) (HS)-SDME mode [22]

2.1.3 Hollow fiber-liquid phase microextraction (HF-LPME)

Hollow fiber-liquid phase microextraction (HF-LPME) is the combination between liquid phase microextraction technique and hollow fiber membrane. Hollow fiber membrane is a porous hydrophobic membrane consisting lots of pores on the wall as shown in Figure 2.3. These pores in the wall provide high contact area between the sample solution and the organic solvent that is supported in the wall of the membrane resulting in high extraction efficiency. Polypropylene hollow fiber membrane with 600 µm internal diameter and 200 µm wall thickness and 0.2 µm pore size, has been widely used in this technique. Figure 2.4 shows typical the HF-LPME setup. HF-LPME consists of a glass vial containing sample solution so call donor solution. The hollow fiber membrane is held at the tip of syringe needles likes U shape. Before extraction process, a piece of hollow fiber membrane is soaked with an organic solvent to fill the pores with the organic solvent, while inside the lumen of hollow fiber contains a micro volume of acceptor solution. The target analytes are extracted from the sample solution through the supported liquid membrane (SLM) and trapped in the extracting solvent, which is filled in the lumen. After extraction, the acceptor solution is drawn by a microsyringe and then injected into the analytical instrument.

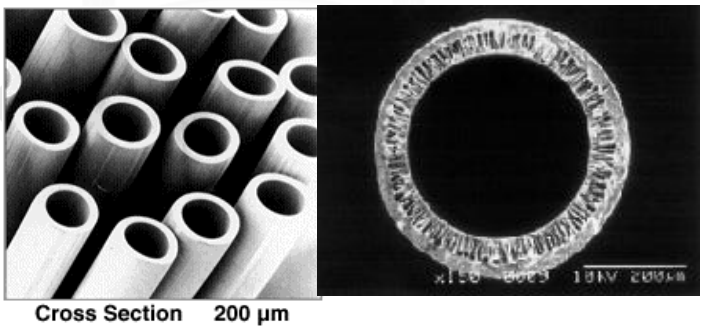

Figure 2.3 Porous hollow fiber membrane [27]

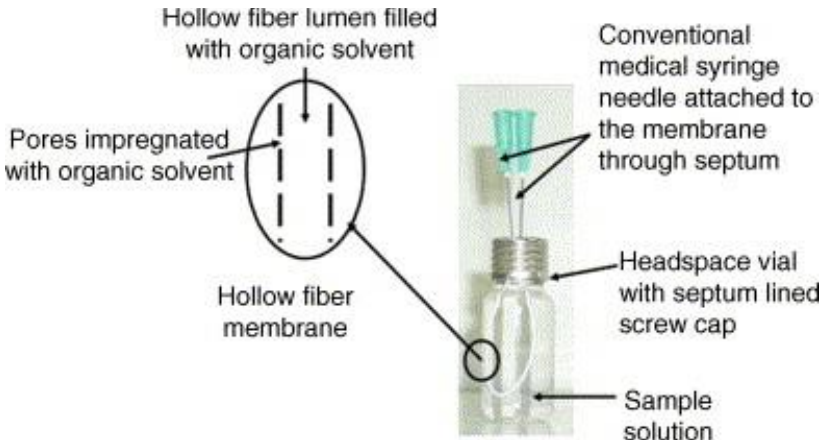

Figure 2.4 Illustration of hollow fiber membrane liquid phase microextraction [28]

The HF-LPME can be operated in two modes; two-phase mode (Figure 2.5 a) and three-phase mode (Figure 2.5 b). In two phase mode, the analytes are extracted from the donor solution (aqueous sample) through the supported liquid membrane (SLM) into the acceptor solution (organic solvent). The reagents in SLM and acceptor solution are the same organic solvent. The two-phase system is suitable for extraction of analytes with high solubility in non-polar organic solvents. The type of transportation is passive diffusion, which depends on the distribution constant ($K_{org/aq}$) of the analytes in donor and acceptor solutions, which is written in Equation 2.2 and the extraction efficiency (EE) can be written in Equation 2.3. In three-phase mode, the analytes are extracted from the donor solution (aqueous sample) through the supported liquid membrane (organic solvent) into the acceptor solution (aqueous solution). The three-phase mode is suitable for extraction of ionizable analyte. The mechanism is based on liquid extraction and back extraction. Generally, the condition in the donor solution is adjusted so that the analytes be present in nonionized form. Then, the analytes are extracted and transferred into the organic solvent (SLM) establishing higher distribution ratio of the analytes in the organic solvent (SLM). After that, the analyte in nonionized form is back extracted into another aqueous solution (acceptor solution) by turning the analyte back into the ionized form. In three-phase HF-LPME, the analyte distributes between the donor phase, organic phase and acceptor phase, which is related to two

equilibriums between organic and donor phase ($K_{o/d}$), and between acceptor and organic phase ($K_{a/o}$) and can be written as the equation below.

A (donor phase) ⇌ A (organic phase) ⇌ A (aqueous acceptor) Equation 2.5

$$K_{a/d} = K_{o/d} \times K_{a/o} = \frac{C_{eq,a}}{C_{eq,d}}$$ Equation 2.6

$$K_{o/d} = \frac{C_{eq,o}}{C_{eq,d}}$$ Equation 2.7

$$K_{a/o} = \frac{C_{eq,a}}{C_{eq,o}}$$ Equation 2.8

where A is the target analyte

$K_{o/d}$ is distribution coefficient of A between the organic phase and donor solution.

$K_{a/o}$ is distribution coefficient of A between the acceptor solution and the organic phase.

$K_{a/d}$ is distribution coefficient of A between the acceptor solution and the donor solution.

$C_{eq,a}$, $C_{eq,o}$ and $C_{eq,d}$ are the concentration of the analyte in the aqueous donor phase, organic phase, and aqueous acceptor solution at equilibrium, respectively.

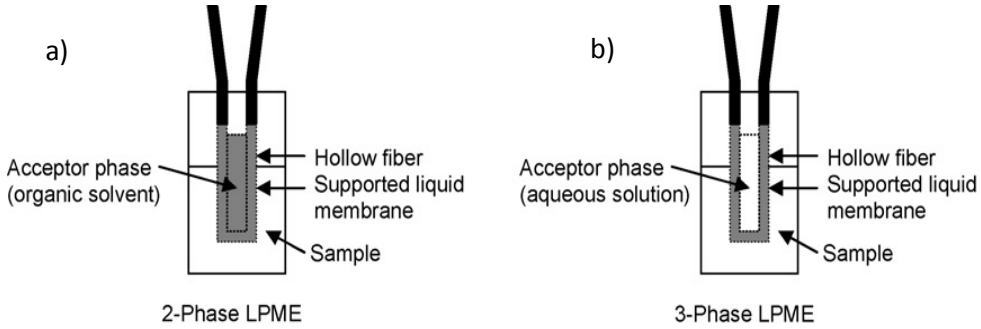

Figure 2.5 Diagrams of HF-LPME systems a) two-phase system and b) three-phase system [29]

Because of the mass transfer of the analyte in HF-LPME is based only on passive diffusion from the donor solution into the acceptor solution, it usually takes a long extraction time in the ranges of 15-60 min [22].

Recently, carbon-nanomaterials has been found to be effective sorption materials due to their large specific surface area and the availability of **π-π** intermolecular interaction.

2.2 Carbon nanomaterials

Carbon nanomaterials have been used as sorbents in variety of applications. In this work, carbon nanomaterials including carbon nanotube, graphene, graphite and carbon nanofibers will be used with hollow fiber membrane by being immobilized in the pores of the membrane serving as analyte trap to facilitate a rapid transportation of analytes and improve the extraction efficiency [30].

2.2.1 Carbon nanotube (CNTs)

Carbon nanotubes (CNTs) are nanomaterials consisting of graphene sheet rolled up into the tubular form [31]. Most of the physical properties of carbon nanotubes derive from graphene and can be divided into a single-walled nanotubes (SWNTs), double-walled nanotubes (DWNTs) and multi-walled nanotubes (MWNTs) as shown in Figure 2.6. The primary advantages of CNTs as sorbent materials are their large surface area, ability to establish **π-π** interactions, and excellent chemical, mechanical and thermal stability. Furthermore, the selectivity of extraction can be tuned by covalently or non-covalently functionalizing the surface of CNTs.

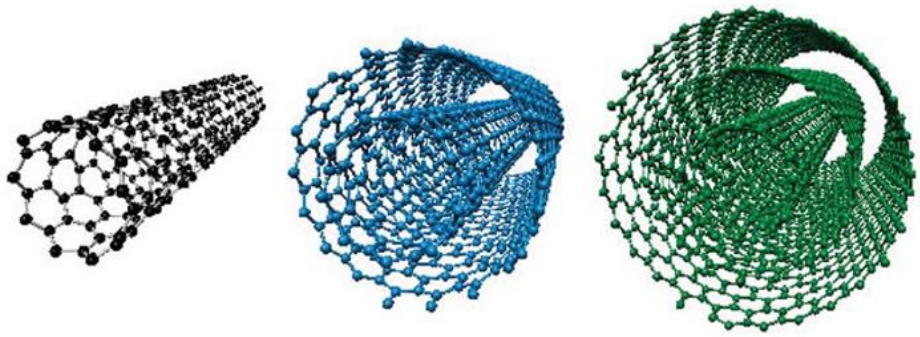

Figure 2.6 Types of carbon nanotubes a) single-walled nanotubes , b) double-walled nanotubes and c) multi-walled nanotubes [32]

2.2.2 Graphene

Graphene is a new form of carbon nanomaterial family, which comprises of a single-layer of sp^2-hybridized carbon atoms arranged in a honeycomb pattern [33]. The structure of graphene is shown in Figure 2.7. Graphene possesses a high theoretical specific surface area and strong adsorption [34]. In addition, due to its large delocalized **π**-electron system, graphene can form a strong **π**-**π** stacking interaction with the benzene ring [35].

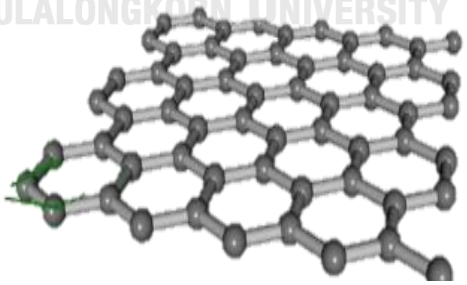

Figure 2.7 Graphene structure [36]

2.2.3 Graphite

Graphite has a planar structure. The layers are stacked parallelly to each other as shown in Figure 2.8. The atoms within the rings are bonded covalently, whilst the layers are loosely bonded together by van der Waals forces. Natural graphite is an excellent conductor of heat and electricity. It is stable over a wide range of temperatures. It is flexible but not elastic and is highly refractory and chemically inert.

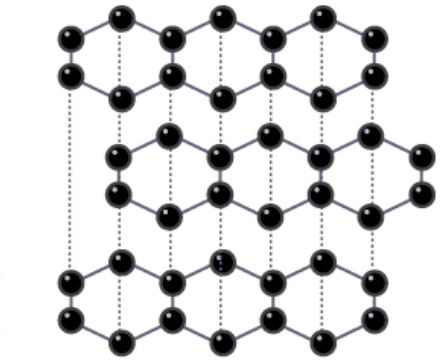

Figure 2.8 Graphite structure [37]

2.2.4 Carbon nanofibers

Carbon nanofibers (CNFs) are cylindrical nanostructures with graphene layers arranged as stacked cones, cups or plates. Figure 2.9 shows the structure of carbon nanofibers. The carbon atoms are bonded together in microscopic crystals. CNFs are very small having average diameters ranging from 125 to 150 nm depending upon the grade, and average lengths ranging from 50 to 100 μm. CNFs have excellent mechanical properties, such as exceptionally high axial strength and large surface area. The properties of CNFs are shown in Table 2.1.

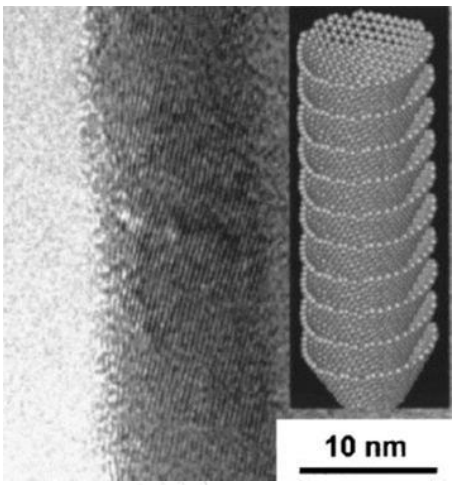

Figure 2.9 Structures of cup-stacked carbon nanofiber under high resolution transmission electron microscope (HRTEM) [38].

Table 2.1 Properties of carbon nanofibers [39]

Property	A	B	C
Outer Diameter (nm)	125 - 150	125 - 150	125 - 150
Inner Diameter (nm)	50-70	50-70	50-70
Specific Surface Area ($m2\ g^{-1}$)	54	39	24
Average pore volume ($cm3\ g^{-1}$)	0.120	0.124	0.075
Average Pore Diameter (angstroms Å)	89.30	126.06	123.99

Where A is carbon nanofiber that pyrotically stripped.
B is carbon nanofiber was treated to 1500°C.
C is carbon nanofiber was treated to 2900°C.

2.3 Trihalomethanes

Trihalomethanes (THMs), including chloroform ($CHCl_3$), bromodichloromethane ($CHBrCl_2$), dibromochloromethane ($CHBr_2Cl$) and bromoform, which are small and relatively polar organic compounds (low K), was selected as model analytes for research I. Trihalomethanes are the prevalent classes of disinfection by-products (DBPs) that are generated during chlorine disinfection process in water treatment plant. The structures of trihalomethanes and their properties are shown in Figure 2.10 and Table 2.2, respectively. THMs are hazardous and some of them are carcinogenic substances leading to DNA damage at low concentration causing various types of cancer [40]. THMs could affect genotoxic mutagens, which can be toxic to humans and aquatic life [41]. The World Health Organization (WHO) recommends that the highest levels of chloroform, dichlorobromomethane, chlorodibromomethane and bromoform allowed in drinking water are 200, 60, 100 and 100 μg L^{-1}, respectively [42].

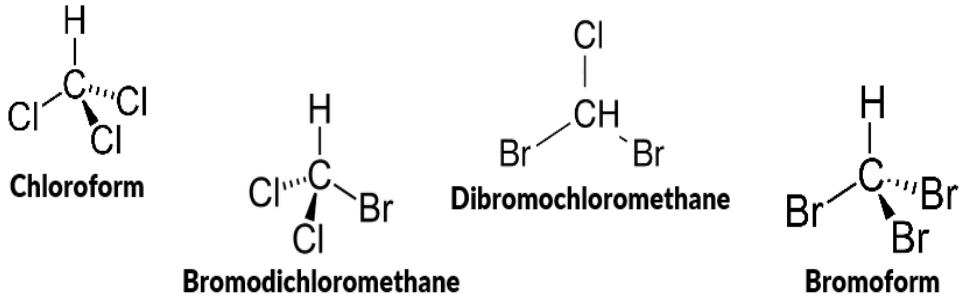

Figure 2.10 Trihalomethanes (THMs) structure; chloroform, bromodichloromethane, dibromochloromethane and bromoform.

Table 2.2 Properties of trihalomethanes (THMs) [43]

Property	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
Molecular weight (g/mol)	119.369	163.823	208.277	252.731
Density (g cm^{-3})	1.48	1.9	2.38	2.89
Boiling Point (°C)	62	90	123-125	149.5
Solubility in water at 20°C (g/100mL)	0.80	0.45	0.27	0.10
LogP	1.97	2.00	2.16	2.40

2.4 Nonsteroidal anti-inflammatory drugs

Non-steroidal anti-inflammatory drugs (NSAIDs), including ketoprofen, naproxen, diclofenac and ibuprofen are pharmaceutical compounds used to treat a various conditions such as headaches, fever, metastatic bone pain and moderate pain caused by inflammation and tissue injury. NSAIDs can cause side effects on humans health such as ulcers in the stomach, aplastic anemia, gastrointestinal disorders and agranulocytosis and changes in renal function [44]. NSAIDs are acidic drugs. Normally, it was administered by oral and 65% of the dose is excreted in the urine. NSAIDs were chosen as model drugs in research II. Table 2.3 and Figure 2.11 are shown the properties and the structure of non-steroidal anti-inflammatory drugs, respectively.

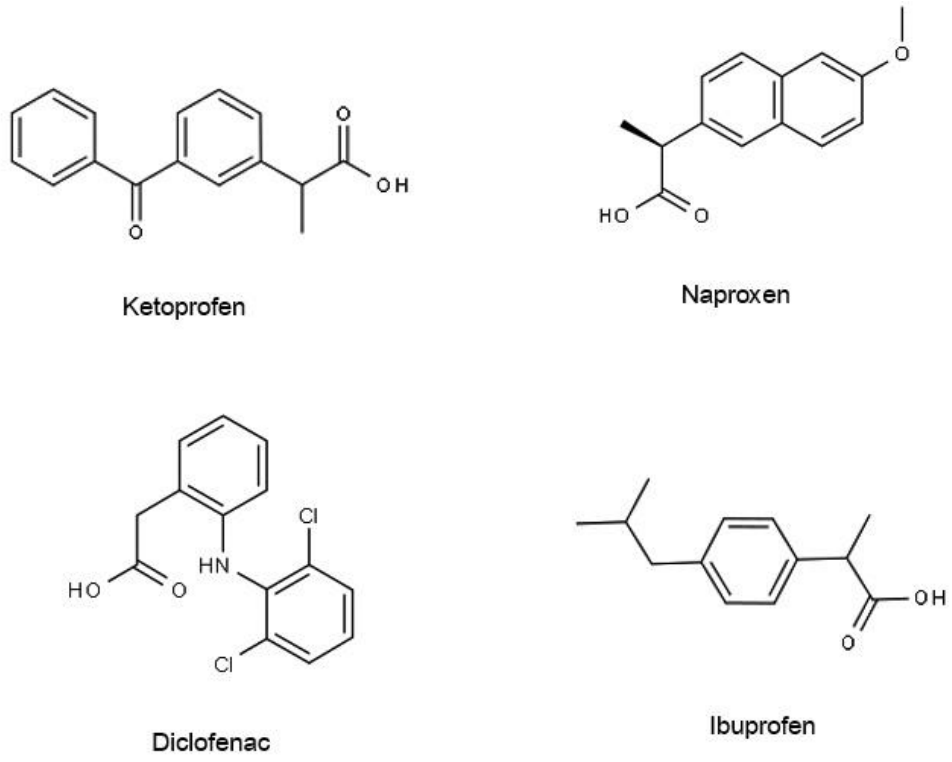

Figure 2.11 Non-steroidal anti-inflammatory drugs (NSAIDs) structure; ketoprofen, naproxen, diclofenac and ibuprofen.

Table 2.3 Properties of non-steroidal anti-inflammatory drugs (NSAIDs). [43]

Property	KET	NAP	DIC	IBU
Molecular weight (g mol^{-1})	254.285	230.263	296.147	206.285
Solubility in water at 25°C (mg L^{-1})	51	15.9	2.37	21
LogP	3.12	3.18	4.51	3.97
pK_a	4.45	4.15	4.15	4.91

CHAPTER III

GRAPHITE REINFORCED HOLLOW FIBER MEMBRANE LIQUID PHASE MICROEXTRACTION FOR DETERMINATION OF TRIHALOMETHANES IN WATER SAMPLES

Abstract

Graphite reinforced hollow fiber liquid phase microextraction was developed for determination of trihalomethanes (THMs) in water samples. The polypropylene hollow fiber was achieved by immobilizing graphite using dispersion in organic solvent. The dispersion was injected into the lumen of conventional HF under pressure and sonicated. The organic solvent as support liquid membrane and the acceptor solvent were 1-octanol. After extraction, the analytes were analyzed using gas chromatography electron captured detector (GC-ECD). The method provided enrichment factors in the range of 40-71 within 10 min extraction time while the enrichment factor of conventional HF-LPME method were 28-62 in 30 min extraction time. The linearity was obtained in the range of 0.2 to 100 $\mu g \cdot L^{-1}$. The limit of detection was below 0.01 $\mu g\ L^{-1}$. The recoveries of spiked THMs at 10 $\mu g \cdot L^{-1}$ in water were between 94 and 111%. The method provided comparative sensitivity to the conventional HF-LPME with shorter extraction time. Finally, the method was applied to real water samples such as drinking water, tap water, and swimming pool water samples.

Keywords: Graphite; hollow fiber liquid phase microextraction; Trihalomethanes; Nanomaterial; Gas chromatography

3.1 Instruments and materials

3.1.1 Polypropylene hollow fiber membrane Accurel® Q3/2, 600 µm ID 200 µm thickness, 0.2 µm pore size (Membrana, Wuppertal, Germany)

3.1.2 Filter membranes (Nylon membrane filter 47 mm 0.45 µm) (Munktell filter, Germany)

3.1.3 Milli-Q ultra-pure water system: model Millipore ZMQS5V00 (Massachusetts, USA)

3.1.4 Ultrasonication bath : model crest575d, Crest Ultrasonic Corporation (New York, USA)

3.1.5 Multi-station magnetic stirrer: model RCT basic IKAMAG®, IKA® Werke GmbH & Co. KG (Staufen, Germany)

3.1.6 Magnetic stirring bars: Spinbar (Wayne, NJ, USA)

3.1.7 pH meter: METTLER TOLEDO (Greifensee, Switzerland)

3.1.8 Microsyringe, 50 µL and 100 µL: Hamilton Company (Nevada, USA)

3.1.9 Medical syringes, 3 mL: Becton Dickinson Medical (S) (Tuas, Singapore)

3.1.10 Medical syringe needles, 500 µm O.D.: Becton Dickinson Medical (S) (Tuas, Singapore)

3.1.11 EPA vial Kit, 20 mL and 30 mL: vertical chromatography (Bangkok, Thailand)

3.1.12 Screw Neck Vial 4 mL, amber glass : La-Pha-Pack (Bangkok, Thailand)

3.1.13 Crimp Neck Vial 1.5 mL, clear glass : La-Pha-Pack (Bangkok, Thailand)

3.1.14 13 mm Combination Seal: PP Screw Cap, black, closed top; Silicone cream/PTFE red, 55° shore A, 1.5 mm: La-Pha-Pack (Bangkok, Thailand)

3.1.15 Crimper 11 mm (MACHEREY-NAGEL, USA)

3.1.16 Decapper 11 mm (MACHEREY-NAGEL, USA)

3.1.17 Micro-Insert clear glass with attached Plastic spring, 0.1 mL: La-Pha-Pack (Bangkok, Thailand)

3.1.18 Autopipettes 10-100 µL, 100-1000 µL, and 1-10 mL (Eppendorf, Germany)

3.1.19 Micropipette tips, 200 µL, 1000 µL and 10 mL (Eppendorf, Germany)

3.1.20 Solvent bottles, 25 mL, 100 mL, 500 mL, and 1000 mL

3.1.21 Beakers, 10 mL, 50 mL, 100 mL, 500 mL, and 1000 mL

3.1.22 Volumetric flasks, 5.00 mL, 10.00 mL, 25.00 mL, 50.00 mL, 100.00 mL, 250.00 mL, 500.00 mL and 1000.00 mL

3.1.23 Agilent 6890N gas chromatograph system with a 63Ni microelectron capture detector (GC-μECD) equipped with an Agilent 7683 autosampler (Agilent Technologies)

3.1.24 Scanning Electron Microscopy (SEM, JEOL, JSM-6480LV)

All glasswares were immersed in 5% HNO_3 and cleaned with detergents and rinsed with deionized water before used.

3.2 Chemicals and reagents

3.2.1 Graphite powder (particle size: 3-4 nm, surface area: 540-650 m^2g^{-1}) : SkySpring Nanomaterials,Inc (Houston, USA)

3.2.2 Graphene powder (diameter: 2 μm, surface area: 750 m^2g^{-1}) : SkySpring Nanomaterials,Inc (Houston, USA)

3.2.3 Multi-walled carbon nanotubes (inner diameter: 5-10 nm, surface area > 60 m^2g^{-1}) : Nanogeneration (Chiang Mai, Thailand)

3.2.4 Trihalomethanes mix standard solution 200 $\mu g \cdot mL^{-1}$ in methanol consists of bromodicloromethane, bromoform, chloroform and dibromochloromethane : Supelco (Bellefonte, PA, USA)

3.2.5 1-octanol (99%): Sigma-Aldrich (St. Louis, MO, USA)

3.2.6 Nitric acid 65%: Merck (Darmstadt, Germany)

3.3 Preparation of chemical solutions

3.3.1 Stock trihalomethanes solution (100 mg·L^{-1})

A 100 mg·L^{-1} stock solution of trihalomethanes was prepared by diluting 200 µg·mL^{-1} of mix standard THMs in 5.00 mL volumetric flask with methanol. The stock standard solution was kept in a refrigerator at 4 ºC before use.

3.3.2 Intermediate standard solution (5 mg·L^{-1})

A 5 mg·L^{-1} intermediate solution of THMs was prepared by diluting of stock standard solution in 10.00 mL volumetric flask with methanol and kept refrigerated in closed glass vial.

3.3.3 Sample solutions (10 µg·L^{-1})

Solutions of 10 µg·L^{-1} THMs in milli-Q water were prepared by diluting intermediate standard solution in 500.00 mL volumetric flask with milli-Q water.

3.4 Experimental

3.4.1 Preparation of carbon sorbent reinforced hollow fiber

The polypropylene hollow fiber membrane was cut manually into 8.0 cm segments. Carbon nanomaterial was dispersed in the organic solvent and directly injected into the lumen and the wall of the hollow fiber membrane and sonicated at room temperature for 2 h to ensure complete immobilization of the carbon nanomaterials in the porous wall. The excess carbon nanomaterial dispersed organic solvent in the lumen was removed with air blow pushed by a medical syringe and washed with the same organic solvent used. Finally, the carbon nanomaterials reinforced hollow fiber membrane was air-dried prior to use.

3.4.2 Hollow fiber liquid phase microextraction procedure

A 30 mL glass vial was used containing 28 mL of THMs sample solution. A 8.0 cm piece of hollow fiber membrane either with carbon sorbent or without carbon sorbent was immersed in an organic solvent as supported liquid membrane (SLM) for 1 minute. The lumen of the membrane was flushed with an air blow for a few times to remove the excess of organic solvent. Then 25 µL of an acceptor solution was filled in the lumen of the hollow fiber membrane using 50 µL Hamilton microsyringe. The setup of hollow fiber liquid phase microextraction is shown in Figure 3.1. After extraction, the acceptor solution remaining in the lumen of hollow fiber membrane was flushed using an air blow from a 5 mL medical syringe and delivered into an insert glass vial placed in 2 mL PTFE/rubber septum crimp GC vial for further analysis.

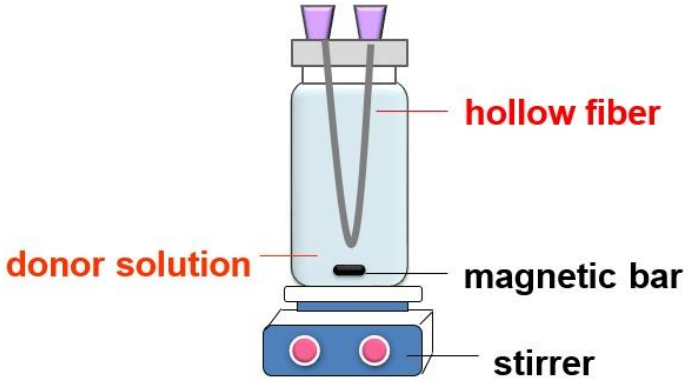

Figure 3.1 Schematic of hollow fiber liquid phase microextraction system

3.4.3 Determination of THMs

Agilent 6890N gas chromatograph system with a 63Ni microelectron capture detector (GC-µECD) equipped with an Agilent 7683 autosampler (Agilent Technologies) was employed for determination of THMs. The HP-5 capillary column, crosslinked (5%-Phenyl)-methylpolysiloxane (30 m × 0.32 mm i.d., 0.25 µm film thickness, J&W Scientific) was used for analysis. Helium (99.999%) was used as the carrier gas with a flow rate of 1.0 mL min^{-1}. Nitrogen (99.999%) was utilized as a makeup gas at

60 mL min^{-1}. The oven temperature was programmed as 40 ℃ for 1 min, increased to 100 ℃ at 5 ℃ min^{-1}, and then increased to 270 ℃ at 20 ℃ min^{-1}. The detector temperature was held at 300 ℃.

3.4.4 Method optimization

Several influential parameters affecting extraction efficiency including types of carbon nanomaterials, organic solvent, and extraction time were examined. The results were reported as enrichment factors in order to evaluate the method efficiency.

3.4.4.1 Types of carbon nanomaterials

Types of carbon nanomaterials are important parameters that affect extraction efficiency. The carbon nanomaterials having large specific surface area such as graphite, graphene, carbon nanotubes reinforced into the hollow fiber membrane were optimized in extraction of THMs. The carbon sorbents reinforced hollow fiber membranes were prepared as mentioned in 3.4.1. In this experiment, 3 mg mL^{-1} of the carbon nanomaterials was dispersed in 1-octanol and 1-octanol was used as the solvent. The extraction performances were compared to the conventional HF-LPME (without carbon reinforcement). The best carbon sorbent will be chosen for the next study.

3.4.4.2 Concentration of carbon sorbent in hollow fiber membrane

The concentration of dispersed carbon sorbent relates to the amount of the carbon sorbent immobilized in the hollow fiber membrane. The effect of the concentration of the dispersed carbon sorbent on the extraction efficiency was optimized. The hollow fiber membranes were soaked in various concentrations of the chosen carbon sorbent in the range 0-5 mg mL^{-1} in 1-octanol.

3.4.4.3 Extraction time

The extraction times in the range of 10-30 min were studied and optimized for reaching the equilibrium distribution process.

3.4.5 Real samples

All drinking water samples were purchased from supermarkets in Bangkok, Thailand. Tap water samples were collected in our laboratory and at author's home. Swimming pool water samples were collected from outdoor swimming pool around author's village and author's residence. The samples were collected in screw cap vials with completely filled and sealed with no headspace. The water samples were filtered through a 0.45 µm nylon membrane filter to remove some particles prior to extraction.

3.5 Method evaluation

3.5.1 Calibration curve and linearity

The calibration curves were plotted between peak areas obtained after extraction and the initial concentrations of standard analytes. Each concentration level was studied at 3 replicates. The linear regression method was used to obtain slope, y-intercept and correlation coefficient (R^2).

3.5.2 Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) refers to the lowest concentration of the analyte that can be detected by the method, while the limit of quantitation (LOQ) is the lowest concentration of the analyte that can be quantitatively determined. The LOD and LOQ were determined based on signal to noise ratio of 3 and 10, respectively.

3.5.3 Enrichment factor

Enrichment factor (EF) is the ratio of the analyte concentration in acceptor solution to its initial concentration in the donor sample. The enrichment factor of the method was calculated according to the Equation 2.4.

3.5.4 Accuracy and precision

Accuracy and precision of the method were evaluated from the spiked samples. The accuracy of the method was expressed by %Recovery as shown in Equation 3.1.

The precision of the method was evaluated by the intra-day relative standard deviation of replicate extractions of spiked samples.

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$$\% \text{ Recovery} = \left(\frac{C_{found} - C_{real}}{C_{added}}\right) X\ 100 \qquad \text{Equation 3.1}$$

Where C_{found} is the concentration of analytes found in spiked sample, C_{real} is the concentration of analytes found in unspiked sample and C_{added} is the concentration of analytes spiked into the sample.

3.6 Results and discussion

3.6.1 Types of carbon nanomaterials

Carbon nanomaterials have been widely used as sorbents because of their properties such as excellent adsorption capacity and high specific surface area. To study the effect of types of carbon nanomaterials, mixed standards at the concentration level of 10 $\mu g \cdot L^{-1}$ were extracted using an 8 cm long hollow fiber membrane for 10 min at 800 rpm. When observed by naked eyes, the surface of modified hollow fiber with graphite appears dark black color. To confirm that nanomaterials were immobilized into the inner wall of hollow fiber, scanning electron microscope (SEM) was used. Figure 3.2 showed the SEM images of a polypropylene hollow fiber, graphene and graphite reinforced hollow fiber at 5000x magnification. The SEM images showed that graphene and graphite have covered the pores of the hollow fiber while unmodified polypropylene hollow fiber membrane showed empty porous surfaces. Figure 3.3 showed the enrichment factors of the four analytes after extraction with HF-LPME reinforced with various types of carbon nanomaterials, graphite, carbon nanotube, graphene and the conventional HF-LPME. As compared to the conventional HF-LPME, the enrichment factors of four analytes in modified hollow fiber membrane were significantly improved, particularly for graphite reinforced hollow

fiber membrane providing the highest enrichment factor. The particle size of graphite powder is the smallest, so it completely immobilized into the lumen of the hollow fiber. According to the results, HF-LPME reinforced with graphite was chosen for further study.

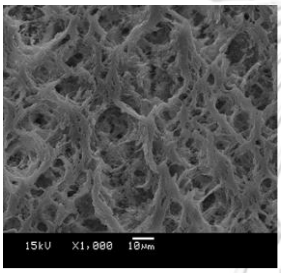
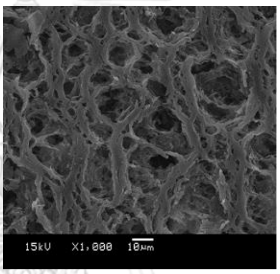
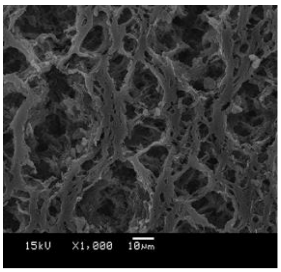
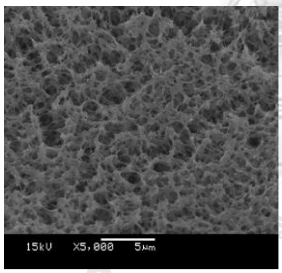
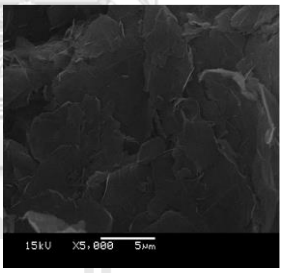
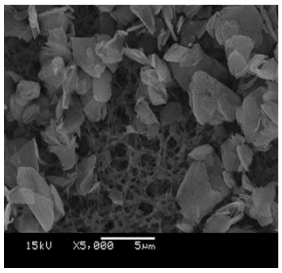
	(A) Polypropylene hollow fiber membrane	(B) Graphene reinforced HF	(C) Graphite reinforced HF
Outside			
Inside			

Figure 3.2 Scanning electron microscope of (A) Polypropylene hollow fiber membrane, (B) graphene-reinforced hollow fiber membrane and (C) graphite-reinforced hollow fiber membrane.

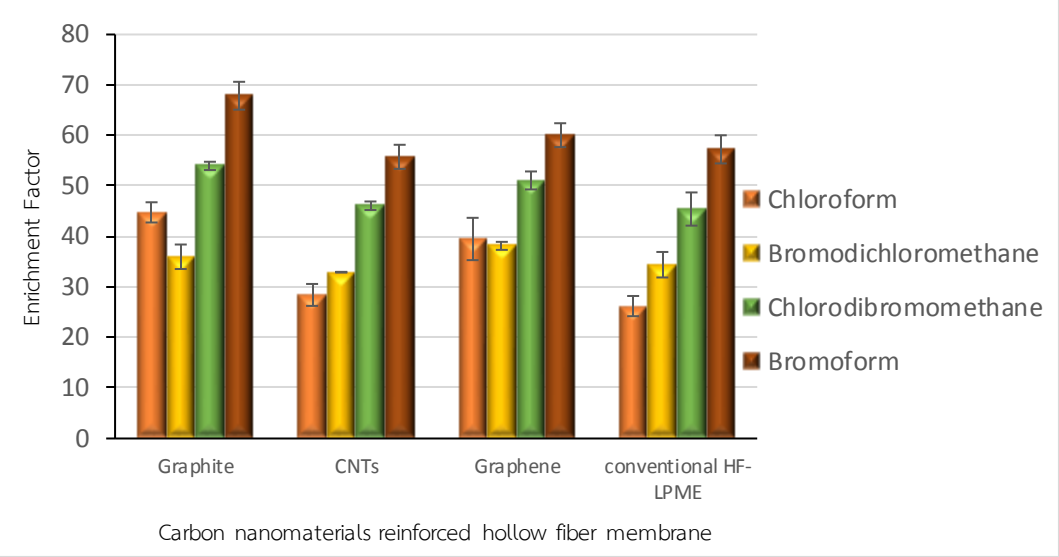


Figure 3.3 Carbon sorbent reinforced hollow fiber membrane microextraction of THMs using various carbon nanomaterials; graphite, carbon nanotube, graphene compared to the conventional HF-LPME. (THMs 10 μg·L^{-1}, acceptor solution: 1-octanol, carbon sorbent 30 mg·mL^{-1}, extraction time : 10 min, stirring speed: 800 rpm)

3.6.2 Concentration of graphite in hollow fiber membrane

In order to provide an additional interaction of graphite into the system, the amounts of graphite loaded into the hollow fiber membrane were optimized in the range of 0-5 mg mL^{-1}. Figure 3.4 shows that the enrichment factor of all analytes increased with an increase in concentration of graphite. At low concentration of graphite, the particles were held in the pores of hollow fiber membrane. At 5 mg mL^{-1}, the EF was decreased due to graphite began to cover the membrane surface and blocked the membrane pores. In this research the optimal concentration of the graphite was 3.0 mg mL^{-1} in 1-octanol.

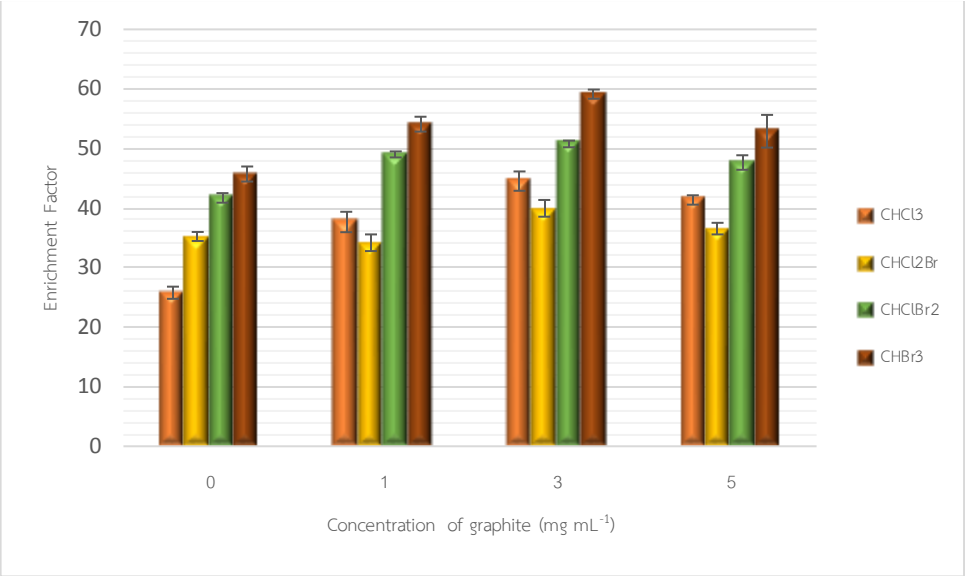


Figure 3.4 Effect of concentration of graphite dispersed in 1-octanol on graphite reinforced HF-LPME of THMs. (THMs 10 μg·L^{-1}, acceptor solution: 1-octanol, extraction time : 10 min, stirring speed: 800 rpm)

3.6.3 Extraction time

HF-LPME is non-exhaustive extraction. The mass transfer is time-dependent process. Extraction time profile is typically established for optimum extraction performance. To study the effect of extraction time, spiked water samples with the concentration of 10 μg·L^{-1} of each standard analyte were extracted at varied extraction time from 5 to 30 min at room temperature. Figure 3.5 showed the extraction time profile of THMs. The enrichment factors for all analytes increased with an increase in extraction time. In case of chloroform, because it is the smallest molecule, it may rapidly transport through the hollow fiber pores and the adsorption process may take place faster than the others. The decreasing of the enrichment factor of chloroform after 10 min might be due to back extraction or adsorption of chloroform from the acceptor solvent to the carbon sorbent dispersed support liquid membrane. The extraction time of 10 min was chosen for the experiment.

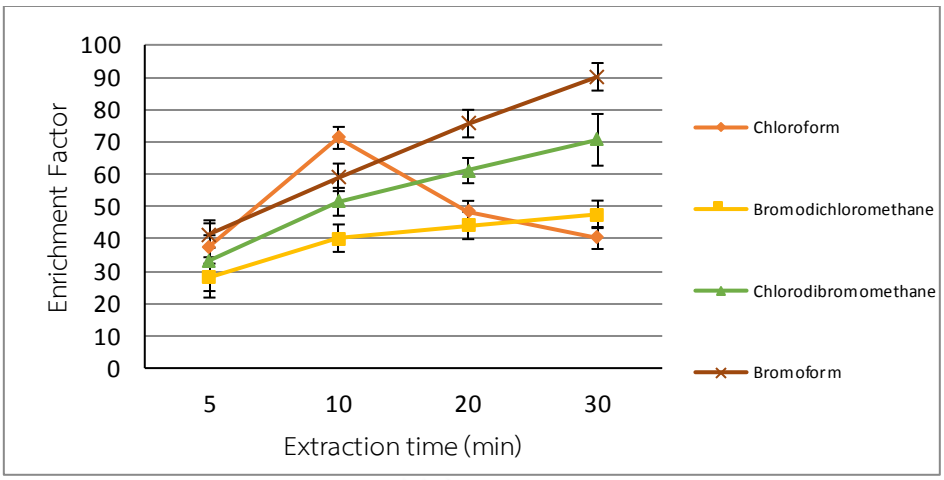


Figure 3.5 Graphite-reinforced hollow fiber membrane microextraction profiles of THMs at various extraction times. (THMs 10 μg·L^{-1}, acceptor solution: 1-octanol, graphite 30 mg·mL^{-1}, stirring speed: 800 rpm)

The conditions of graphite reinforced HF-LPME method for determination of THMs in water samples are summarized in Table 3.1.

Table 3.1 Conditions of HF-LPME for determination of THMs in water samples

EME parameters	Optimum condition
Hollow fiber length	8 cm
Organic solvent	1-octanol
Donor solution	Water sample
Donor volume	28 mL
Acceptor solution	1-octanol
Acceptor volume	25 μL
Extraction time	10 min
Stirring speed	800 rpm

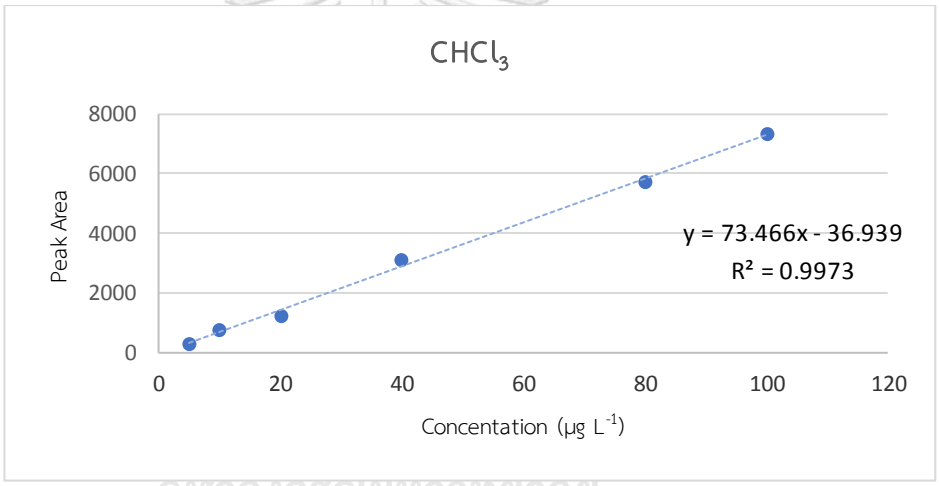
3.7 Method evaluation

The HF-LPME method for determination of THMs was evaluated for its analytical merits using mixed standard samples solution in milli-Q water.

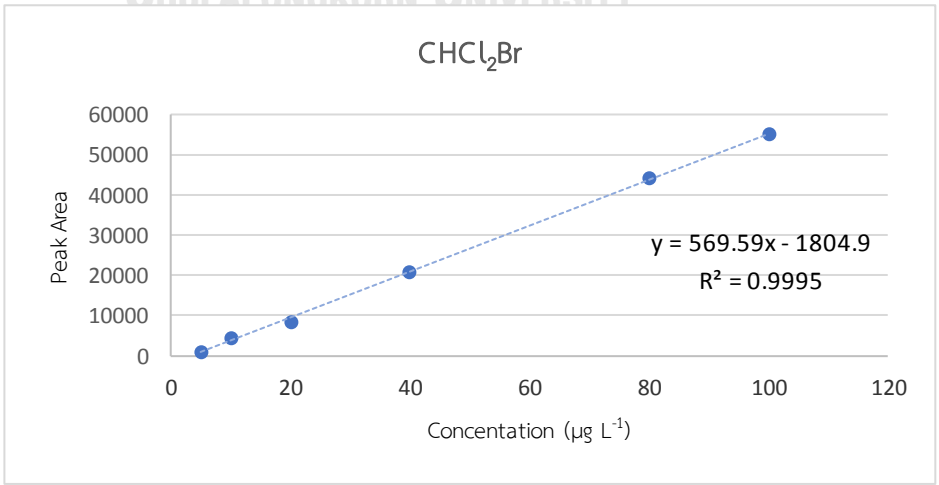
3.7.1 Calibration curve and linearity

The calibration curve of each THMs was established in the range of 0.20-100.0 $\mu g \cdot L^{-1}$ as shown in Figure 3.6. Good linearity was obtained over the working range with coefficient of determination (R^2) greater than 0.9953.

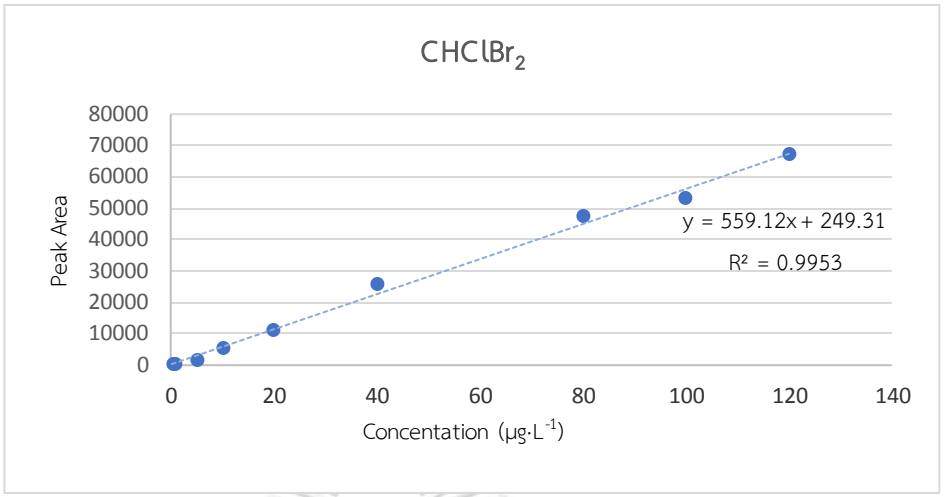
(A)



(B)



(C)



(D)

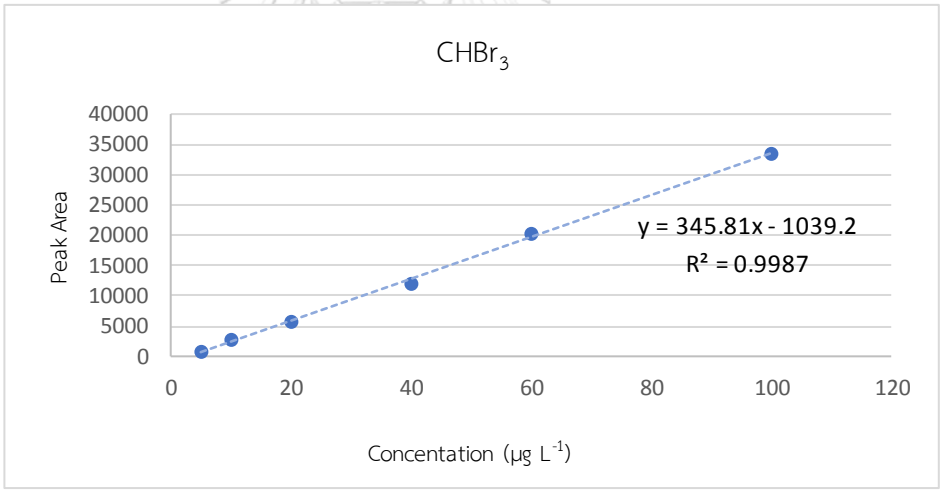


Figure 3.6 Calibration curve of graphite reinforced HF-LPME method for determination of THMs from water samples (A) trichloromethane (B) bromodichloromethane (C) chlorodibromomethane and (D) bromoform.

3.7.2 Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of the method were calculated based on signal to noise ratio of 3 and 10, respectively. The method LOD and LOQ for determination of THMs from water samples are reported in Table 3.2. The LOD and LOQ values are in low $\mu g \cdot L^{-1}$ level, which are below the maximum contaminant levels (MCLs) of THMs in drinking water and tap water (200 $\mu g \cdot L^{-1}$, 60 $\mu g \cdot L^{-1}$, 100 $\mu g \cdot L^{-1}$, 100 $\mu g \cdot L^{-1}$ for $CHCl_3$, CHC_2Br, $CHClBr_2$ and $CHBr_3$ respectively) recommended by World Health Organization (WHO). This method can be applied for determination of THMs in drinking waters.

3.7.3 Enrichment factor

The enrichment factor of the graphite reinforced HF-LPME method for determination of THMs were in the range of 40-71 (Table 3.2), calculated from the final concentration of THMs in the acceptor to the spiked concentration of THMs the donor solution or in the sample (Equation 2.4). The extraction efficiencies calculated from the Equation 2.3 were in the range of 0.04-0.06.

3.7.4 Accuracy and precision

The accuracy of the method was expressed as %recovery of spiked THMs in water. In this research, replicate extractions of spiked 10 $\mu g \cdot L^{-1}$ of THMs water samples were analyzed and calculated from the equation 3.1. The results showed that percent recoveries of trihalomethanes standard solution were in the range of 94-111% as shown in Table 3.2. The method repeatability evaluated from relative standard deviations (%RSD) of concentration. The results are reported in Table 3.2. The intra-day precision (n=5) of this method was less than 2.1%. It indicates that the developed method provides good precision.

Table 3.2 Analytical performance of graphite reinforced hollow fiber liquid phase microextraction for determination of THMs in milli-Q water samples.

Compound	Linear range ($\mu g \cdot L^{-1}$)	R^2	LOD ($\mu g \cdot L^{-1}$)	LOQ ($\mu g \cdot L^{-1}$)	EF	%Recovery (10 $\mu g \cdot L^{-1}$)	% RSD (n=5)
$CHCl_3$	0.2-100	0.9973	0.10	0.50	71	103	2.1
$CHCl_2Br$	0.2-100	0.9995	0.01	0.04	40	111	1.3
$CHClBr_2$	0.2-120	0.9953	0.01	0.05	51	94	1.6
$CHBr_3$	0.2-100	0.9987	0.05	0.20	59	106	1.6

3.8 Real samples

The method was applied for determination of THMs from several sources of water samples such as drinking water, tap water and swimming pool water samples. The concentrations of THMs found in real water samples are summarized in Table 3.3. It can be seen that the concentrations of THMs found in all drinking water samples were below the maximum contaminant levels (MCLs) values of WHO. For swimming pool water sample, the concentration of chloroform was very high due to the reaction of disinfection chlorine with organic compounds.

Table 3.3 THMs concentrations found in drinking water, tap water and swimming pool water samples

Sample	Concentration ($\mu g \cdot L^{-1}$) (RSD%)			
	$CHCl_3$	$CHCl_2Br$	$CHClBr_2$	$CHBr_3$
Milli-Q water	ND	ND	ND	ND
Mineral water	ND	ND	ND	ND
Drinking water 1	ND	11.68 (2.45)	2.45 (1.83)	ND
Drinking water 2	ND	ND	0.22 (3.21)	5.11 (2.05)
Drinking water 2 + spiked THMs (10 $\mu g \cdot L^{-1}$)	10.93 (1.79)	11.81 (3.13)	13.56 (1.57)	15.55 (2.91)
Drinking water 3	ND	4.99 (3.22)	0.94 (3.22)	3.14 (0.59)
Drinking water 4	ND	3.33 (0.57)	0.24 (2.25)	3.04 (0.25)
Tap water 1	66.88 (1.89)	8.05 (2.12)	6.81 (2.63)	5.61 (1.74)
Tap water 2	48.44 (1.13)	21.76 (3.17)	12.85 (3.01)	3.88 (2.24)
Swimming pool water 1	152.35 (2.17)	9.06 (2.14)	2.11 (2.63)	3.19 (0.90)
Swimming pool water 2	221.81 (1.96)	29.35 (2.86)	0.73 (2.87)	3.15 (0.37)

ND = non detected.

3.9 Method comparison

The method for determination of THMs in water samples were compared between our method and the conventional LPME method [28]. The comparison were summarized in Table 3.4. The analytical performance of this method is equivalent to the previous method, but graphite-hollow fiber membrane provided shorter extraction time than the conventional HF-LPME. This phenomenon might be due to the addition of graphite in the hollow fiber membrane that facilitate rapid mass transfer of THMs through the pores of the hollow fiber membrane resulting in shorter extraction time.

Table 3.4 Comparison of our method with the previous method

	Hollow fiber	Extraction time (min)	Enrichment factor	Linear range	LOD ($\mu g \cdot L^{-1}$)
Our method	Graphite reinforced hollow fiber	10	40-71	0.2-100	0.01-0.1
Previous method	Polypropylene hollow fiber	30	28-62	0.2-100	0.01-0.2

3.10 Conclusion

Carbon nanomaterials have been studied to reinforce into hollow fiber membrane to improve the extraction efficiency of HF-LPME of small and relatively polar organic compounds such as THMs. The graphite reinforced HF-LPME method was developed for extraction of THMs and applied to real water samples such as drinking water, tap water, and swimming pool water samples. The addition of graphite in the pores of the hollow fiber membrane serves as an analyte trap providing an additional mechanism to enhance solute transportation resulting to improvement of the extraction efficiency and provides higher enrichment factor and shorter analysis time

than the conventional HF-LPME. The optimal conditions for extraction of THMs were 1-octanol as supported liquid organic solvent and acceptor solution, 10 min extraction time and 800 rpm stirring rate. The analytical performance of this method provided a good linearity with the working range from 0.20 to 100.00 $\mu g \cdot L^{-1}$. Accuracy and precision are in the acceptable range.

CHAPTER IV

DEVELOPMENT OF AN AUTOMATIC NANOMATERIALS REINFORCED HOLLOW FIBER MEMBRANE LIQUID PHASE MICROEXTRACTION COMBINED WITH HPLC FOR DETERMINATION OF ACIDIC DRUGS IN URINE

Abstract

An automatic carbon nanofiber reinforced hollow fiber liquid phase microextraction combined with HPLC was developed for the determination of nonsteroidal anti-inflammatory drug in urine samples. The carbon nanofiber was dispersed in organic solvent and immobilized in polypropylene hollow fiber membrane. The HF-LPME extraction chamber was designed and fabricated by 3D printer and configured to connect to HPLC system. Parameters affecting extraction efficiency including organic solvent as supported liquid membrane, donor and acceptor condition, and analytical sequence of automatic extraction and HPLC determination were investigated and optimized. The analytes were extracted in the 3D-printed module. The acceptor solution was sequently carried to HPLC interface for neutralization of the acceptor solution prior to injection into the HPLC system. The method was fully automated providing total analysis time of 47 min and the sample throughput of 4 samples hr^{-1}. The limit of detections for the method were ranged from 1.6-5.6 $\mu g\ L^{-1}$. The recoveries in real human urine samples were in the range of 97-105 % and the relative standard deviations were between 0.3-4.6 % (n=5). This technique showed improved results compared to the conventional HF-LPME.

Keywords: carbon nannofiber; hollow fiber liquid phase microextraction; non-steroidal anti-inflammatory drugs (NSAIDs); automatic; high performance liquid chromatography; urine samples

4.1 Instruments and materials

4.1.1 Polypropylene hollow fiber membrane Accurel® Q3/2, 600 µm ID 200 µm thickness, 0.2 µm pore size (Membrana, Wuppertal, Germany)

4.1.2 Filter membranes (Nylon membrane filter 47 mm 0.45 µm) (Munktell filter, Germany)

4.1.3 Milli-Q ultra-pure water system: model Millipore ZMQS5V00 (Massachusetts, USA)

4.1.4 Ultrasonication bath : model crest575d, Crest Ultrasonic Corporation (New York, USA)

4.1.5 Multi-station magnetic stirrer: model RCT basic IKAMAG®, IKA® Werke GmbH & Co. KG (Staufen, Germany)

4.1.6 Magnetic stirring bars: Spinbar (Wayne, NJ, USA)

4.1.7 pH meter: METTLER TOLEDO (Greifensee, Switzerland)

4.1.8 Microsyringe, 50 µL : Hamilton Company (Nevada, USA)

4.1.9 Medical syringes, 3 mL: Becton Dickinson Medical (S) (Tuas, Singapore)

4.1.10 Medical syringe needles, 500 µm O.D.: Becton Dickinson Medical (S) (Tuas, Singapore)

4.1.11 EPA vial Kit, 20 mL and 30 mL: vertical chromatography (Bangkok, Thailand)

4.1.12 Screw Neck Vial 4 mL, amber glass : La-Pha-Pack (Bangkok, Thailand)

4.1.13 13 mm Combination Seal: PP Screw Cap, black, closed top; Silicone cream/PTFE red, 55° shore A, 1.5 mm: La-Pha-Pack (Bangkok, Thailand)

4.1.14 Micro-Insert clear glass with attached Plastic spring, 0.1 mL: La-Pha-Pack (Bangkok, Thailand)

4.1.15 Autopipettes 10-100 µL, 100-1000 µL, and 1-10 mL (Eppendorf, Germany)

4.1.16 Micropipette tips, 200 µL, 1000 µL and 10 mL (Eppendorf, Germany)

4.1.17 Solvent bottles, 25 mL, 100 mL, 500 mL, and 1000 mL

4.1.18 Beakers, 10 mL, 50 mL, 100 mL, 500 mL, and 1000 mL

4.1.19 Volumetric flasks, 5.00 mL, 10.00 mL, 25.00 mL, 50.00 mL, 100.00 mL, 250.00 mL, 500.00 mL and 1000.00 mL

4.1.20 Xcalibur syringe pump : Cavro (Sunnyvale, USA)

4.1.21 1 mL gastight glass syringe : Hamilton (Bonaduz, Switzerland)

4.1.22 2 mL holding coil of PTFE tubing : IDEX (Oak Harbor, USA)

4.1.23 3D printer (Formlabs)

4.1.24 16W low pressure Hg lamp UV oven (KA-9180, PSKY, China)

4.1.25 The liquid chromatographic system composed of a quaternary high-pressure pump (PU-4180) equipped with autosampler (Jasco, Tokyo, Japan)

4.1.26 C18 core-shell reversed-phase column (Phenomenex, Torrance, US)

4.1.27 Scanning Electron Microscopy (SEM, JEOL, JSM-6480LV)

All glasswares were immersed in 5% HNO_3 and cleaned with detergents and rinsed with deionized water before used.

4.2 Chemicals and reagents

4.2.1 Graphite powder (particle size: 3-4 nm, surface area: 540-650 m^2g^{-1}) : SkySpring Nanomaterials,Inc (Houston, USA)

4.2.2 Graphene powder (diameter: 2 μm, surface area: 750 m^2g^{-1}) : SkySpring Nanomaterials,Inc (Houston, USA)

4.2.3 Multi-walled carbon nanotubes (inner diameter: 5-10 nm, surface area > 60 m^2g^{-1}) : Nanogeneration (Chiang Mai, Thailand)

4.2.4 Multi-walled carbon nanotubes functionalised with 5% of -COOH (diameter: 10 nm, average length: 1-2 μm) : IIIDropSens (Spain)

4.2.5 Carbon nanofibers (diameter: 70-150 nm, surface area: 40 m^2g^{-1}) : Electrovac AG (Klosterneuburg, Austria)

4.2.6 Ketoprofen, Ibuprofen, Naproxen, Diclofenac sodium salt solid standard (≥98%) : Sigma-Aldrich (Madrid, Spain)

4.2.7 2-(2,4,5-Trichlorophenoxy)propionic acid (also termed silvex or fenoprop) solid standard : Sigma-Aldrich (Madrid, Spain)

4.2.8 Sodium phosphate dibasic/sodium phosphate monobasic buffer solution pH 8 : Sigma-Aldrich (Madrid, Spain)

4.2.9 Boric acid/potassium chloride/Sodium hydroxide buffer solution pH 10: (Scharlau)

4.2.10 Methanol : Merck (Darmstadt, Germany)

4.2.11 1-octanol (99%): Sigma-Aldrich (St. Louis, MO, USA)

4.2.12 Dihexyl ether (97%) : Sigma-Aldrich (St. Louis, MO, USA)

4.2.13 Nitric acid 65%: Merck (Darmstadt, Germany)

4.2.14 Hydrochloric acid 36.5%: Merck (Darmstadt, Germany)

4.2.15 Formic acid 98-100% : Merck (Darmstadt, Germany)

4.2.16 Sodium hydroxide (≥99%): Merck (Darmstadt, Germany)

4.3 Preparation of chemical solutions

4.3.1 Stock NSAIDs solution (100 mg·L^{-1})

A 100 mg·L^{-1} stock solution of ketoprofen, ibuprofen, naproxen and diclofenac was prepared from solid standard dissolved in methanol. The stock standard solution was stored in the refrigerator at 4 ºC before use.

4.3.2 Working standard solution (30 µg·L^{-1})

A 30 µg·L^{-1} of working standard solution was prepared by diluting of stock standard solution with Milli-Q water in 250.00 mL volumetric flask and adjusted the pH to 2.0 using dilute HCl.

4.3.3 Internal standard solution (40 mg·L^{-1})

A 40 mg·L^{-1} of 2-(2,4,5-Trichlorophenoxy)propionic acid was prepared by dissolving 0.004 g of 2-(2,4,5-Trichlorophenoxy)propionic acid in 100.00 mL volumetric flask with milli-Q water.

4.3.4 Sodium hydroxide (20 mmol·L^{-1})

A 20 mmol·L^{-1} of NaOH was prepared by dissolving 0.0800 g of NaOH in 100.00 mL volumetric flask with milli-Q water.

4.3.5 Hydrochloric acid (20 mmol·L^{-1})

The 1.0 mol·L^{-1} HCl was prepared by pipetting 2.08 mL of concentrated HCl solution in 25.00 mL volumetric flask with milli-Q water. A 20.0 mmol·L^{-1} of HCl was diluted with milli-Q water by pipetting 2.00 mL of 1.0 mol·L^{-1} HCl into a 10.00 mL volumetric flask.

4.3.6 Buffer solutions (10 mmol·L^{-1})

Buffer solutions of pH 8, and pH 10 at the 10 mmol·L^{-1} were prepared from sodium phosphate dibasic/sodium phosphate monobasic and boric acid, respectively, using appropriate amounts of sodium hydroxide for pH adjustment.

4.4 Experimental

4.4.1 Preparation of carbon nanomaterials reinforced hollow fiber

The polypropylene hollow fiber membrane was cut manually into 12.0 cm segments. A 3 mg mL-1 of carbon nanomaterials was dispersed in the organic solvent and directly injected into the lumen and the wall of hollow fiber membrane and sonicated at room temperature for 1 h to ensure complete immobilization of carbon nanomaterials in the porous wall. The excess carbon nanomaterials dispersed organic solvent in the lumen was carefully removed with air blow pushed by a medical syringe and washed with the same organic solvent used. Finally, the carbon nanomaterials reinforced hollow fiber membrane was air-dried prior to use.

4.4.2 3D printed LPME microextraction chamber

The 3D printed extraction chamber was designed using the 123D Design Software. It consisted of a 12 cm long cylinder, 3mm ID and 2 mm wall thickness (see Figure 4.1). Two additional connections allowed to perfuse the donor compartment and were

placed at 1 cm distance of the cylinder ends, that is 10 cm apart, serving as donor inlet and outlet, respectively. The 3D model was exported in .stl file format and transferred to the PreForm software prior to 3D printing. The model was tilted 80° from the vertical and fabricated with 503 layers at 100 µm z-resolution. Other features of the 3D print were as follows: automatic supports with density = 1, point size = 0.6 mm, no internal supports, flat spacing =5mm, slope multiplier = 1, base thickness = 2 mm and height above base = 5 mm. After printing, the chambers were separated from the built platform, immersed in isopropyl alcohol for 10 min, followed by removal of the polymeric supports, and curing of the printed platforms overnight in a 16W low pressure Hg lamp UV oven.

Figure 4.1 Sketch of a 3D flow through chamber model for in-line HF-LPME

4.4.3 In-line carbon sorbent reinforced HF-LPME coupled with HPLC system

A 12.0-cm carbon sorbent reinforced hollow fiber membrane was inserted through the 3D printed extraction chamber, fitted into short fluorinated ethylene propylene (FEP) tubing sleeves of 1.07 mm ID. Both ends of the hollow fiber membrane were sealed with inverted ferrules and nuts. The acceptor solution was pumped into the lumen of the hollow fiber membrane while the donor solution was fed to the upper nut into the extraction chamber and flowed outside the membrane through the chamber. The front end of the extraction chamber was connected to an eight-port multiposition selection valve, which all lines are linked with the central port of the selection vale via the polytetrafluoroethylene (PTFE) holding coil. The selection valve allowed to further manipulate the samples, solvent, acceptor phase or air through the

holding coil. The outlet of the extraction chamber was connected to a three-way solenoid valve furnished with 1.0 mL gas-tight glass syringe for pumping of HCl to neutralize the eluent prior to filling the HPLC loop. Figure 4.2 shows the diagram of the fully automatic in-line hollow fiber liquid phase microextraction coupled with HPLC.

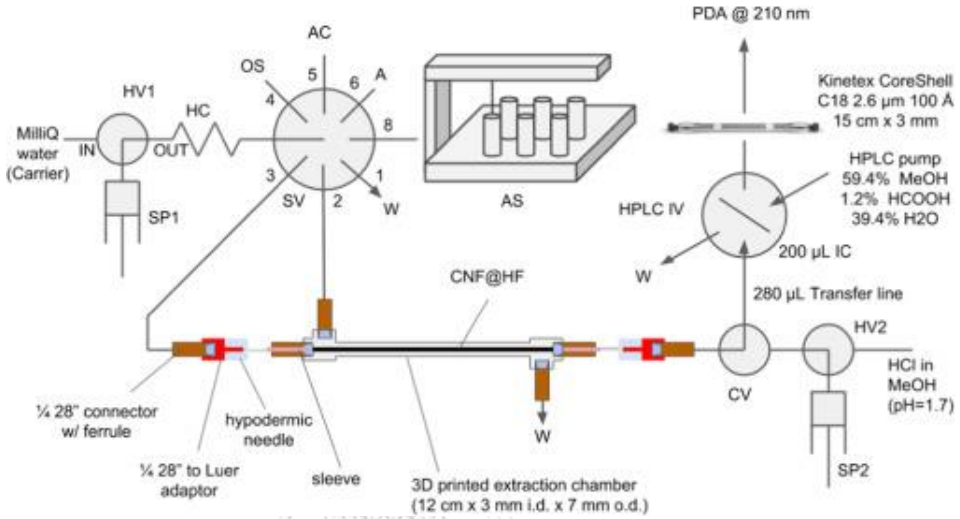


Figure 4.2 Schematic diagram of the fully automatic in-line extraction using carbon sorbent reinforced HF-LPME coupled to HPLC system.

4.4.4 Analytical sequence for in-line carbon sorbent reinforced HF-LPME coupled to HPLC system

Membrane pretreatment

Step 1, the carrier solution (20 mM HCl) was filled into the donor chamber to avoid organic solvent leaking while filling into the lumen of hollow fiber membrane. A 200 µL of acceptor solution (20 mM NaOH) and 60 µL organic solvent (dihexyl ether) were loaded in holding coil, respectively (step2-3). Fill the organic solvent into the lumen of hollow fiber membrane and wait 60s to ensure that the pores were completely filled (step 4-5). The excess organic solvent in the lumen was removed by

flushed with acceptor solution 180 µL (step 6). This acceptor solution was ready in the lumen of hollow fiber for the extraction process.

Removal of organic solvent

A 500 µL of acidic methanol was aspirated into the external syringe and pumped into the HPLC line to remove the organic solvent that was used as supporting liquid membrane. (step7-8)

Extraction procedure

A 800 µL of sample solution was loaded in the holding coil and fed into the donor chamber at the flow rate of 0.5 mL min^{-1}. This process was repeated 8 times (step 9-10). The total volume of the sample was 6.4 mL.

Neutralization and collection of the sample solution

A 220 µL of acceptor solution was loaded in the holding coil and 220 µL of 20 mM HCl was loaded into the syringe, and both of them are mixed simultaneously by pumping with solenoid valve in the ratio of 1:1 within 11 times before entering the HPLC injector. (step 11-14)

Cleaning the pores of hollow fiber membrane

The lumen of the hollow fiber membrane was rinsed with the acceptor solution to minimize analyte carryover and striped out much of the liquid membrane prior to the next analysis (step 15-16).

The overall of analytical sequence of the automatic in-line hollow fiber liquid phase microextraction coupled with HPLC are summarized in Table 4.1.

Table 4.1 Analytical sequence of the automatic CNF@HF LPME/SPME method

Step	Operational step	Direction	SV Position	Flow rate (mL min^{-1})	Volume (µL)
Membrane pretreatment					
1	Fill the donor chamber with carrier solution	Dispense	2	0.5	800
2	Draw the acceptor solution into the holding coil	Aspirate	5	1.0	200
3	Draw the organic solvent into the holding coil	Aspirate	4	1.0	60
4	Fill the lumen of the hollow fiber membrane with the organic solvent	Dispense	3	0.3	60
5	Wait (60s)				
6	Flush the organic solvent and trap the acceptor solution into the HF lumen	Dispense	3	0.3	180
Removal of organic solvent					
7	Fill external syringe with 20 mM HCl in MeOH	Aspirate	---	1.0	500
8	Pump 20 mM HCl in MeOH into HPLC line	Dispense	---	1.0	500

	Operational step	Direction	SV Position	Flow rate (mL min^{-1})	Volume (μL)
Microextraction procedure					
Loop start (8 times)					
9	Draw the sample into holding coil	Aspirate	8	1.0	800
10	Fill the sample into donor compartment and wait(30s)	Dispense	2	0.5	800
Loop end					
Neutralization and collection of extract					
11	Draw the acceptor solution into holding coil	Aspirate	5	1.0	220
12	Fill external syringe with 20mM HCl	Aspirate	---	1.0	220
	Loop start (11 times)				
13	Pump extract/acceptor into HPLC line	Dispense	3	0.3	20
14	Pump 20mM HCl into HPLC line	Dispense	---	0.3	20
	End Loop				
Cleaning the pore of hollow fiber membrane					
15	Draw the acceptor solution into the holding coil	Aspirate	5	1.0	80
16	Fill the lumen of the hollow fiber membrane with acceptor solution	Dispense	3	0.3	80

4.4.5 HPLC Instrumentation and on-line interface

The liquid chromatographic module system is composed of a quaternary high-pressure pump equipped with a high pressure injection valve furnished with 1/32'' ID stainless steel sample loop of 200 µL; an external GECKO 2000 column heater; a photo-diode array detector, and a C18 core-shell reversed-phase column preceded by a C18 SecurityGuard™ Standard precolumn for the separation of the target species. Isocratic elution with MeOH : H_2O : HCOOH in the ratio 59.4:39.4:1.2 (v/v/v) at the flow rate of 0.40 mL min^{-1} was used. The temperature was kept at 30℃ throughout. Detection of the analytes were accomplished at 210 nm and 230 nm for ibuprofen. The retention times of ketoprofen, naproxen, internal standard, diclofenac and ibuprofen under the selected experimental conditions were 4.4, 5.4, 11.3, 14.8 and 17.0 min, respectively.

4.4.6 Method optimization

4.4.6.1 Types of carbon nanomaterials

The carbon nanomaterials having a large specific surface area such as graphite, graphene, multi-walled carbon nanotubes (MWCNTs), oxidized MWCNTs, and carbon nanfibers (CNFs) reinforced into the hollow fiber membrane were studied and optimized in extraction of NSAIDs in batch mode. The carbon sorbents reinforced hollow fiber membranes were prepared as mentioned in 4.4.1. In this experiment, 3 mg mL^{-1} of the carbon nanomaterials was dispersed in 1-octanol and 1-octanol was used as the solvent. The extraction performances were compared to the conventional HF-LPME (without carbon reinforcement). The best carbon sorbent will be chosen for the next study.

4.4.6.2 Types of organic solvents

The organic solvent should have suitable properties such as low vapor pressure, low viscosity and should be compatible with the detection system. In this work, 1-octanol and dihexyl ether were optimized.

4.4.6.3 pH of donor and acceptor solution

A suitable pH value of the acceptor solution and donor solution can improve the extraction efficiency. Since the target analytes are acidic drugs, the pH value of donor solution could affect forms of the drugs and transportation ability across the liquid membrane. The pH of the donor sample should be adjusted to below analyte pK_a in order to keep the analytes in non-dissociated forms so that they can be extracted and transported across the organic liquid membrane while the acceptor solution is adjusted to be alkaline in order to deprotonate the analytes into dissociated forms so that they cannot be back extracted and transported back to the donor solution. In this work, the sample pH was adjusted to pH 2 using HCl. The pH of the acceptor solution was adjusted to pH 8, 10 and 12 using sodium phosphate dibasic/sodium phosphate monobasic, boric acid buffer solution and NaOH, respectively.

4.4.6.4 Extraction chamber length.

The extraction chamber length may affect the extraction efficiency of the method by allowing more contact area. In this work, chamber length at 8, 12 and 16 cm were optimized.

4.4.6.5 Real samples

Urine samples were collected from a healthy 26 years old female volunteer and a 42 years old male volunteer. The urine samples were filtered through a 0.45 um membrane filter to remove suspended matter. The volunteers received a single oral administration of a given drug indicated in results and discussion. A time-course sample collection was resorted to the investigation of drug clearance. A blank urine sample was obtained from the same volunteers before oral administration.

4.5 Method evaluation

4.5.1 Calibration curve and linearity

The calibration curves were plotted between peak areas obtained after extraction and the initial concentrations of standard analytes. Each concentration level was studied at 3 replicates. The linear regression method was used to obtain slope, y-intercept and correlation coefficient (R^2).

4.5.2 Limit of detection (LOD) and limit of quantification (LOQ)

The limit of detection (LOD) refers to the lowest concentration of the analyte that can be detected by the method, while the limit of quantitation (LOQ) is the lowest concentration of the analyte that can be quantitatively determined. The LOD and LOQ were determined based on signal to noise ratio of 3 and 10, respectively.

4.5.3 Enrichment factor

Enrichment factor (EF) is the ratio of the analyte concentration in acceptor solution to its initial concentration in the donor sample. The enrichment factor of the method was calculated according to the Equation 2.4.

4.5.4 Accuracy and precision

Accuracy and precision of the method were evaluated from the spiked samples. The accuracy of the method was expressed by %Recovery as shown in Equation 3.1. The precision of the method was evaluated by the intra-day relative standard deviation of replicate extractions of spiked samples.

4.5.5 Relative Recovery

Relative recovery was calculated by comparing the mean area response of extracted samples (spiked before extraction) to that of unextracted samples (spiked after extraction) at each concentration level.

4.6 Results and discussion

4.6.1 Types of carbon nanomaterials

Preliminary tests were conducted in a batchwise mode to investigate the feasibility of a three phase hybrid SPE-LPME method for extraction of acidic drugs. To study the effect of types of carbon nanomaterials, spiked water samples with the concentration of 30 $\mu g \cdot L^{-1}$ of each standard analyte were extracted at 10 min extraction time and 800 rpm. Figure 4.3 showed the enrichment factor of the four analytes obtained from carbon sorbent HF-LPME using various types of carbon nanomaterials; graphite, graphene, carbon nanotube, functionalized carbon nanotube, carbon nanofiber and compared to the conventional HF-LPME. The hollow fiber reinforced with carbon nanofiber gave higher enrichment factor among the other carbon reinforced HF-LPME and the conventional HF-LPME. In conventional HF-LPME, analyte extraction occurs through the pores of a hollow fiber; where the extractant is immobilized. The mechanism is based on only passive diffusion whereas immobilization of carbon nanomaterials in the pores of the hollow fiber membrane can improve mass transfer, increase the selectivity providing additional mechanism resulting in increased extraction efficiency and enrichment factor [45]. Carbon nanofiber provided the highest enrichment factor because of the structure of the carbon nanofiber is like a cup-stacked, which has many reactive edges both inside and outside [46]. Therefore, carbon nanofibers were chosen for the remained of the work in a carbon sorbent reinforced HF-LPME mode. Scanning electron micrographic (SEM) images shown in Figure 4.4 demonstrated the carbon nanofiber in the pores of the inner wall and the shell side of the hollow fiber membrane.

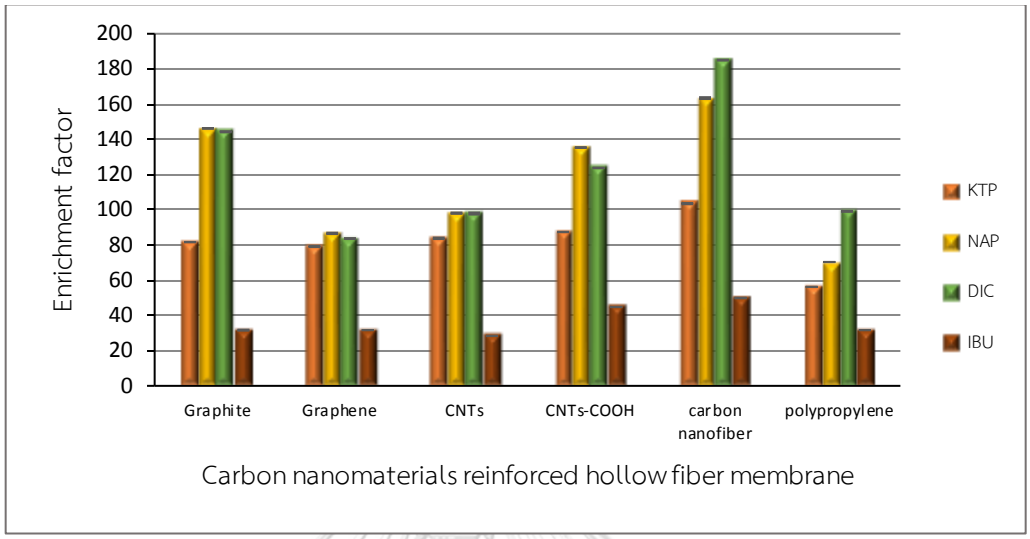


Figure 4.3 Carbon nanomaterial reinforced hollow fiber membrane microextraction profiles of NSAIDs using various carbon nanomaterials; graphite, graphene, carbon nanotube, functionalized carbon nanotube with -COOH, carbon nanofiber and polypropylene hollow fiber. (Donor solution: NSAIDs 30 $\mu g \cdot L^{-1}$ adjusted to pH 1.7, acceptor solution: 20mM NaOH, SLM: dihexyl ether, carbon sorbent: 30 $mg \cdot mL^{-1}$, stirring speed: 1000 rpm)

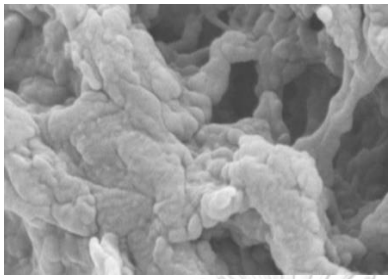
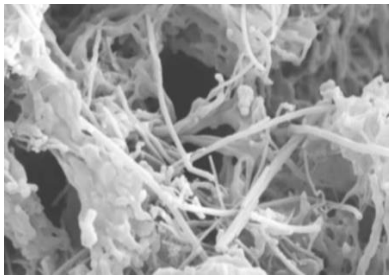
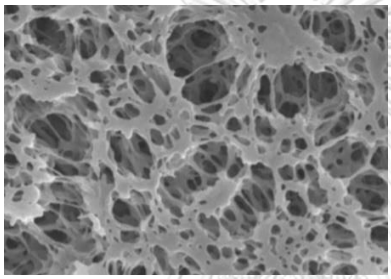
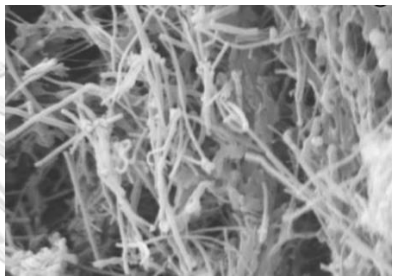
	(A) Polypropylene hollow fiber membrane	(B) Carbon nanofiber reinforced HF
Outside		
Inside		

Figure 4.4 Scanning electronic micrographs at 10k magnification illustrating the polypropylene hollow fiber (A) polypropylene hollow fiber membrane (B) carbon nanofiber reinforced hollow fiber membrane.

4.6.2 Types of organic solvent

Due to carbon nanofiber and polypropylene membrane are hydrophobic in nature and low wettability; therefore slow extraction rate and low extraction efficiency was obtained. To address these issues supported organic solvent was used. The property of the organic solvent immobilized in the pores of the hollow fiber such as the viscosity might affect the extraction efficiency. The organic solvent should have a low volatility to prevent evaporation and volatile loss, appropriate viscosity to make a rapid mass transfer and also solvent should provide high distribution constants for the analytes. Based on previous works reported in the literature for NSAIDs [47-49] , 1-octanol and dihexyl ether were evaluated. Preliminary tests were conducted in a batchwise mode

to investigate the feasibility of carbon sorbent reinforced hollow fiber membrane for extraction of acidic drugs. A 25.0 mL of spiked 30 μg·L^{-1} acidic drugs in water, the extraction time and stirring rate were 10 min and 800 rpm, respectively. The results are shown in Figure 4.5. Dihexyl ether bearing the lowest viscosity (1.7mPas vs 7.3mPas for 1-octanol at 20℃) and the lowest dielectric constant (<2.7 vs 10.3 for 1-octanol at 20℃) afforded better distribution constants of the drugs from the CNFs into the solvent, and fostered a significant increase of enrichment factor for the two more hydrophobic species (viz., DIC and IBU) by 31% and 67%, respectively, as compared to 1-octanol. Therefore, dihexyl ether was chosen as the support liquid membrane for carbon sorbent reinforced HF-LPME.

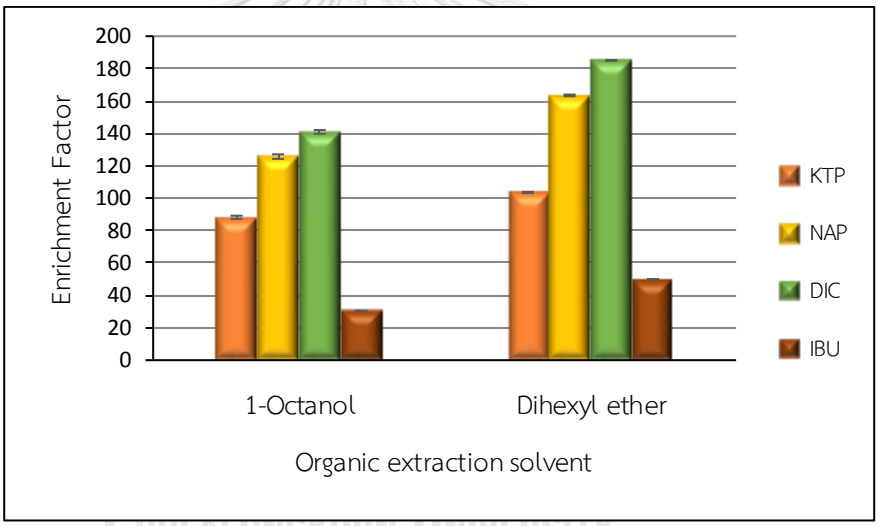


Figure 4.5 Effect of organic solvent on extraction efficiencies of NSAIDs.
(Donor solution: NSAIDs 30 μg·L^{-1} adjusted to pH 1.7, acceptor solution: 20mM NaOH, carbon nanofiber: 30 mg·mL^{-1}, stirring speed: 1000 rpm)

4.6.3 pH of donor and acceptor solution

Due to the pK_a values for KTP, NAP, DIC and IBU is 4.45, 4.15, 4.15 and 4.91, respectively. The pH value of donor solution should be adjusted to acidity keeping the analytes in non-dissociated form while the acceptor solution should be adjusted to basicity for deprotonation of the analytes desorbing them from the carbon sorbent

dispersed supported liquid membrane into the acceptor solution. Preliminary tests were conducted in a batchwise. To study the effect of pH in acceptor solution, spiked water samples with the concentration of 30 $\mu g \cdot L^{-1}$ of each standard analyte and the pH of the acceptor varied at pH 8.1, 10.0 and 12.3 with 10 $mmol \cdot L^{-1}$ PBS buffer, and borate buffer and 20 $mmol \cdot L^{-1}$ NaOH, respectively were optimized. Figure 4.6 showed the concentration of the four analytes in acceptor solution. The concentration for all analytes increased with an increase in pH of acceptor solution. The results showed similar results to the previous research that determination of ibuprofen, diclofenac and salicylic acid using HF-LPME and detected by HPLC [17, 50, 51]. Hence, pH 2.0 HCl in the donor phase and pH 12.3 with a concentration of 20 $mmol \cdot L^{-1}$ NaOH in the acceptor phase were selected. To circumvent analyte carryover for concentration levels above 300 $\mu g \cdot L^{-1}$ as a result of **π-π** stacking interactions with the CNFs, 10% (v/v) MeOH was added to the alkaline acceptor. Hence, the donor and acceptor phases were composed of 10 $mmol \cdot L^{-1}$ HCl and 10% (v/v) MeOH in 20 $mmol \cdot L^{-1}$ NaOH, respectively.

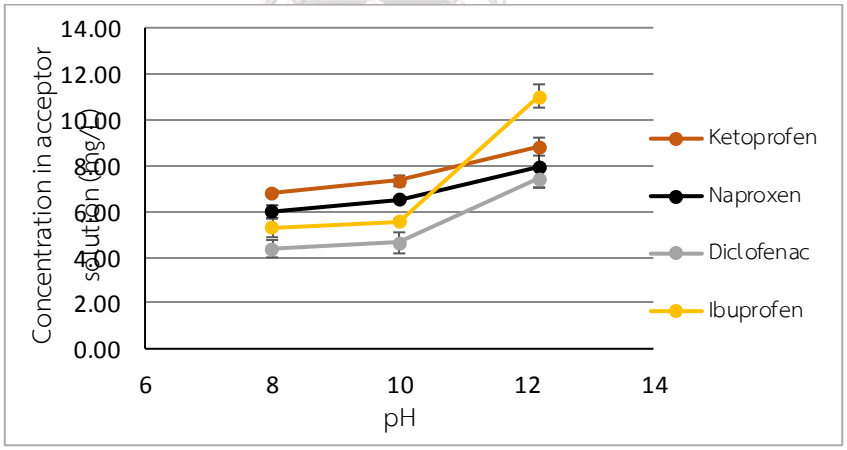


Figure 4.6 Effect of acceptor pH on carbon nanofiber reinforced hollow fiber membrane microextraction extraction of NSAIDs. (Donor solution: NSAIDs 30 $\mu g \cdot L^{-1}$ adjusted to pH 1.7, carbon nanofiber: 30 $mg \cdot mL^{-1}$, stirring speed: 1000 rpm)

4.6.4 Extraction chamber length

To study the effect of donor chamber size, spiked water samples with the concentration of 30 μg·L^{-1} of each standard analyte and the length of chamber varied at 8, 12 and 16 cm were optimized. It can be seen in Figure 4.7 that the enrichment factor of NSAIDs when extracted with 12-cm extraction chamber provided the highest enrichment factor. For shorter chamber, the contact area between the hollow fiber membrane and the donor solution was limited while for longer chamber, the enrichment factor was lower because of the leaking of the organic solvent and acceptor solution during extraction process due to the built-up of backpressure has been occasionally observed during the automatic flow-through microextraction process. Therefore, the 12 cm of donor chamber was used in this experiment.

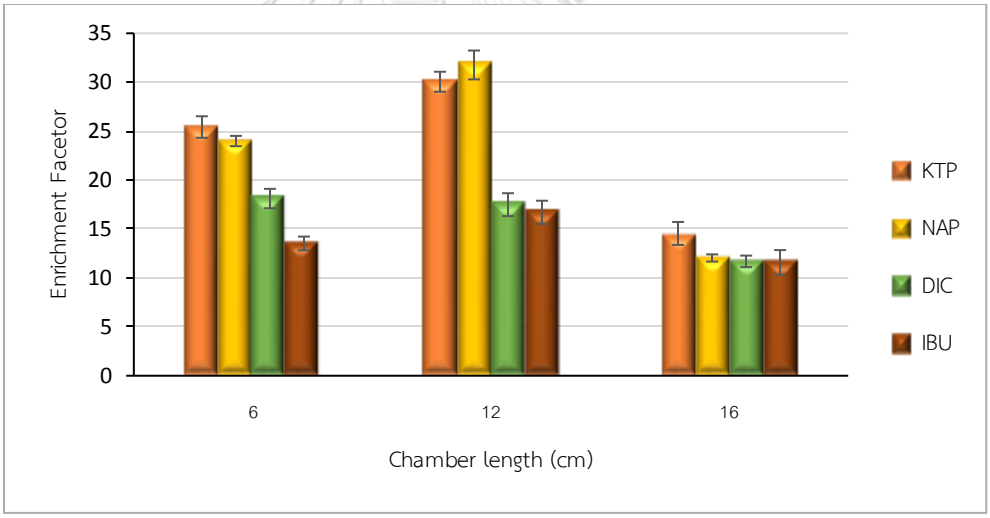


Figure 4.7 Effect of extraction chamber length. (Donor solution: NSAIDs 30 μg·L^{-1} adjusted to pH 1.7, acceptor solution: 20mM NaOH, carbon nanofiber: 30 mg·mL^{-1})

4.6.5 Evaluation of experimental parameters for in-line CNF@LPME

To evaluate the online method, it is important to optimize the sample volume in the donor chamber, flow rate and stop time in each extraction. To study this effect, 800-6400 µL of sample solution corresponding to 1-8 loops of aspirate sample solution into the donor chamber, flow rate of 500-1000 µL min^{-1} and stop time of 10-30 s were optimized. To prevent the HPLC column, Since the acceptor solution after extraction is alkaline, so it should be neutralized with dilute acid prior to entering the HPLC system to prevent the HPLC column from damage. The volume of mixing between basic extract and acidic solution was studied at 9-13 loops. The best results were summarized in Table 4.2.

Table 4.2 Conditions of in-line CNF@LPME for determination of NSAIDs.

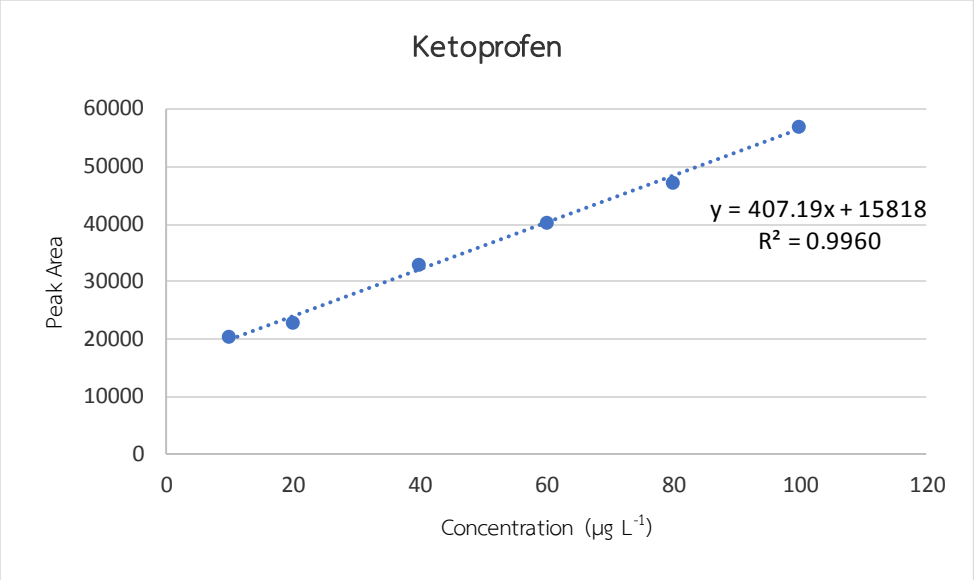
Parameter	Value
Sample volume (µL)	800
Number of aspirate sample solution into the donor chamber (loops)	8
Sample flow rate (µL min^{-1})	500
Stop time (s)	30
Number of mixing between basic and acidic solution (loops)	11

4.7 Method evaluation

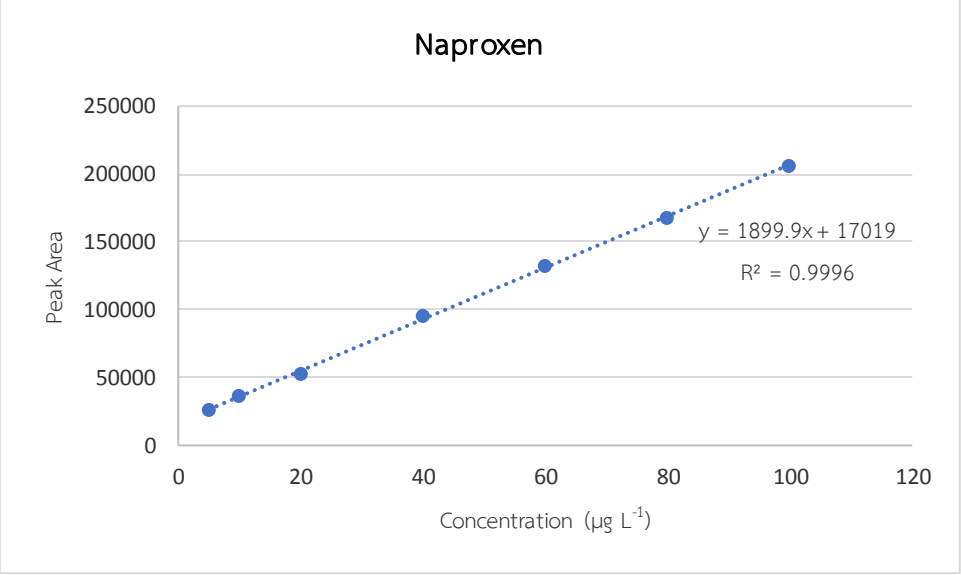
4.7.1 Calibration curve and linearity

The calibration curve of each THMs was established in the range of 10.00 -100.00 µg·L^{-1} as shown in Figure 4.8. Good linearity was obtained over the working range with coefficient of determination (R^2) greater than 0.9960. The chromatograms of spiked NSAIDs in water samples extraction via carbon nanofiber reinforced hollow fiber membrane-LPME/HPLC-DAD see in Figure 4.9.

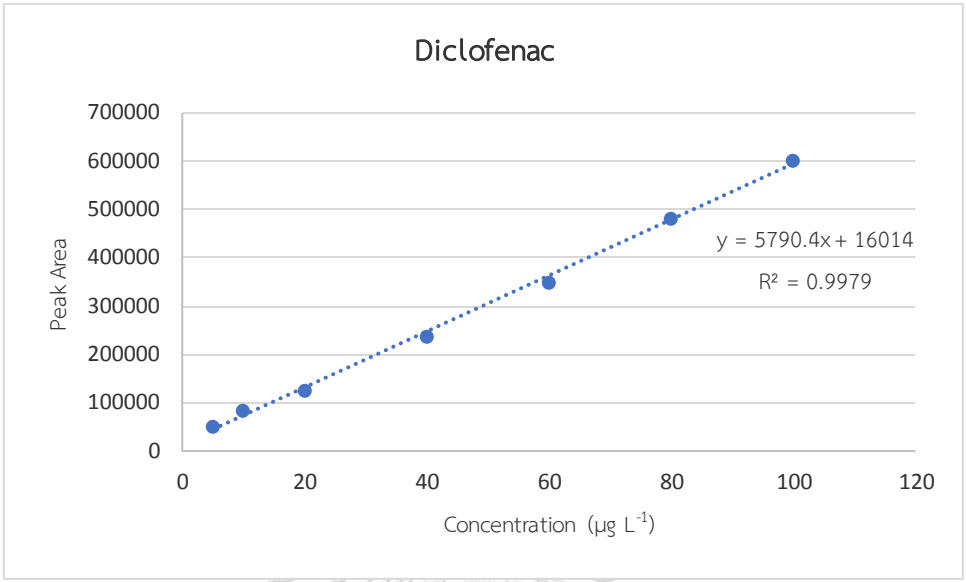
(A)


Ketoprofen
Peak Area
60000
50000
40000
30000
20000
10000
0
0
20
40
60
80
100
120
Concentration (µg L-1)
y = 407.19x + 15818
R² = 0.9960

(B)


Naproxen
Peak Area
250000
200000
150000
100000
50000
0
0
20
40
60
80
100
120
Concentration (µg L-1)
y = 1899.9x + 17019
R² = 0.9996

(C)



(D)

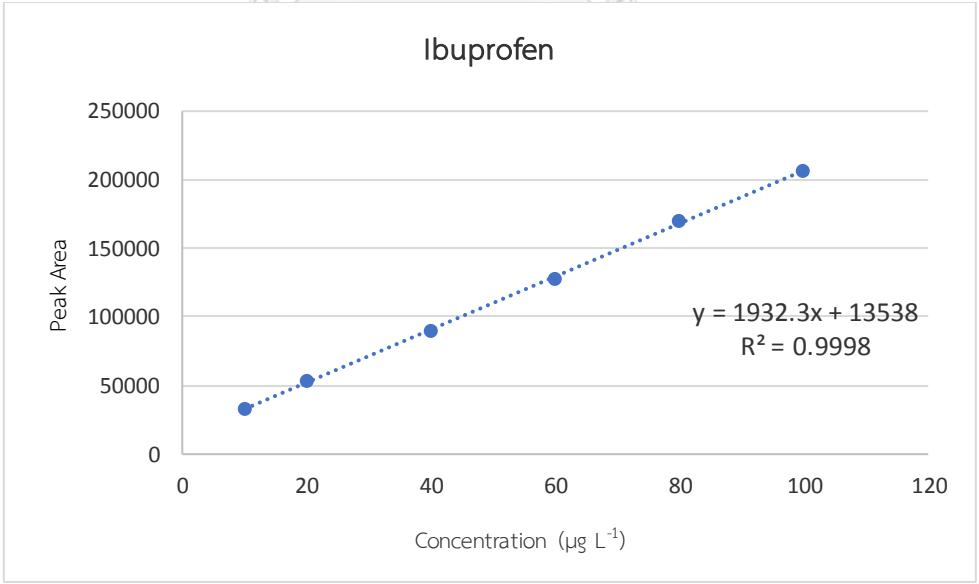


Figure 4.8 Working range of NSAIDs determined by in-line CNF@LPME hyphenated to LC. (A) ketoprofen, (B) naproxen, (C) diclofenac and (D) ibuprofen.

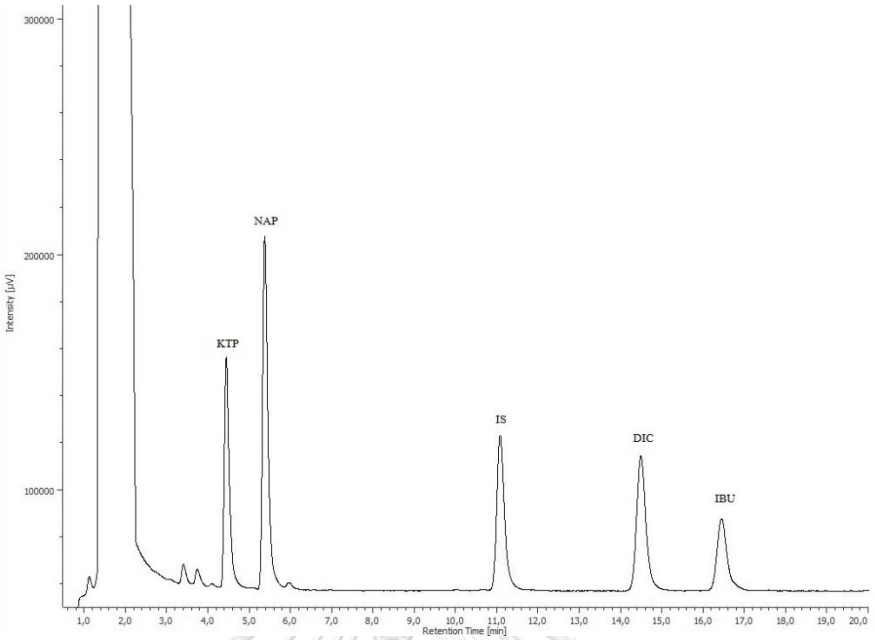


Figure 4.9 Representative carbon nanofiber reinforced hollow fiber membrane - LPME/HPLC-DAD chromatograms of spiked NSAIDs at a concentration level of 80 mg L^{-1} of each drugs and internal standard.

4.7.2 Matrix effect

The proposed method was applied to urine samples. The extraction performances differed from water sample matrices. Therefore, methods of matrix-match calibration method and standard addition method are recommended for accurate results. Calibration curves for real samples were established in urine in the range of 10 to 500 µg·L^{-1}. (see Appendix Figure A.1). Good linearity was obtained with coefficient of determination (R^2) greater than 0.9992.

4.7.3 Limit of detection (LOD) and limit of quantification (LOQ)

The LOD and LOQ of the method were calculated based on signal to noise ratio of 3 and 10, respectively. The method LOD and LOQ for determination of NSAIDs from urine samples are reported in Table 4.3. The LOD and LOQ values are in low µg·L^{-1} level.

4.7.4 Enrichment factor

The enrichment factor of the carbon nanofiber reinforced LPME hyphenated to LC method for determination of NSAIDs were in the range 43.2-96.8 (Table 4.3), calculated from the final concentration of NSAIDs in the acceptor to the spiked concentration of NSAIDs the donor solution or in the sample (Equation 2.4).

4.7.5 Accuracy and Precision

The accuracy of the method was expressed as %recovery of spiked NSAIDs in urine samples. In this research, replicate extractions of spiked three concentration levels of NSAIDs in urine samples were analyzed and calculated from the equation 3.2. The result showed that percent recoveries of NSAIDs were in the range of 94-111% as shown in Table 4.4. The method repeatability evaluated from relative standard deviations (%RSD) of concentration. The results are reported in Table 4.3. The intra-day precision (n=5) of this method was less than 6.1%. It indicates that the developed method provides good precision.

Table 4.3 Analytical performance of the carbon nanofiber reinforced LPME hyphenated to LC method for determination of NSAIDs.

Compound	Linear range ($\mu g \cdot L^{-1}$)	R^2	LOD ($\mu g \cdot L^{-1}$)	LOQ ($\mu g \cdot L^{-1}$)	EF	RSD% (n=5)
KET	10-500	0.9998	2.2	7.4	55	6.1
NAP	5-500	0.9997	1.6	5.3	71	4.9
DIC	5-500	0.9992	3.7	12.4	82	3.7
IBU	10-500	0.9993	4.3	14.4	41	5.9

4.8 Real Samples

In our study, the established method successfully quantified NSAIDs after oral administration. Urine samples were collected from two healthy volunteers and analyzed using the developed extraction method with HPLC analysis. To suppress matrix effects and to prevent contamination of the fiber, the sample are diluted with milli-Q water in the ratio 1:3 and filtered through a nylon membrane filter. Their pH values were adjusted at 2.0 by addition of HCl solutions. Figure 4.10 showed the HPLC chromatograms of blank of human urine sample and human urine samples after the administration of drugs. The chromatograms show excellent baselines with peak absence. Table 4.4 showed the concentrations of NSAIDs found in real human urine samples. It is clear that this method is applicable for the monitoring of acidic drugs in real human urine samples.

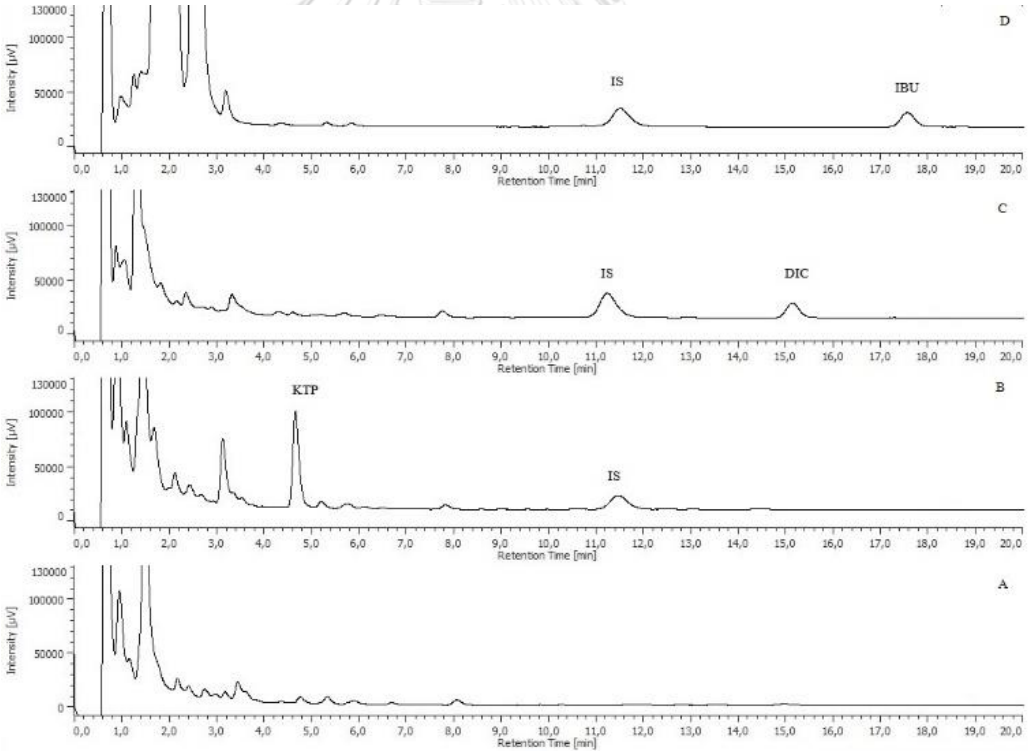


Figure 4.10 Representative carbon nanofiber reinforced hollow fiber membrane - LPME/HPLC-DAD chromatograms of blank urine (A) and of urine obtained after the administration of an oral dose of ketoprofen (B), diclofenac (C) and ibuprofen (D).

Table 4.4 Acidic drugs concentrations in real urine sample after the administration of oral doses.

Sample	Administration		Sampling time (min)[b]	Concen-tration ($\mu g \cdot L^{-1}$)	R (%)	RSD (%) (n=5)
	NSAID	Dosage[a]				
Urine 1	KTP	50	60	160.12		0.32
Urine 2	KTP	50	240	198.70		3.17
Urine 3	DIC	100	60	57.60		1.54
Urine 4	IBU	600	240	79.20		4.59
Urine 2 + spiked KTP (100 $\mu g \cdot L^{-1}$)				297.87	100	1.51
Urine 3 + spiked DIC (30 $\mu g \cdot L^{-1}$)				87.57	96.7	2.09
Urine 4 + spiked IBU (40 $\mu g \cdot L^{-1}$)				121.38	105	1.68

[a] Amount of drug administrated via oral (in mg)

[b] Sampling time after administration (min)

4.9 Method comparison

The comparison between our method and the previous method on extraction of acidic drugs is summarized in Table 4.5. All of the papers surveyed are based on manual/semi-automatic operation of the LPME setup with off-line analysis of the extracts [9, 13, 17, 50, 51]. From the table, the use of CNF provided better sensitivity and dynamic linear range with LODs more than 10-fold compared with a conventional HF-LPME method [50]. LODs in our work are also on a par of those previously reported for electromembrane extraction [17, 52, 53], and the analytical performance showed that the LODs of both techniques provided the same range but this work used lower sample volume and no need of resorting to external energy sources.

Table 4.5 Comparison of our method with the previous method reported in the literature for determination of NSAIDs.

Method	Sample preparation	Analytes	Sample volume (mL)	Recovery (%)	Linear range ($\mu g \cdot L^{-1}$)	LOD ($\mu g \cdot L^{-1}$)	Ref.
LC/UV-Vis	HF-LPME	IBU, DIC, SAC	50	83-99	135-10,000	40-53	[50]
LC/UV-Vis	EME	SAC, KTR, KTP, NAP, DIC, IBU	10	58-100	0.29-100	0.08-3.36	[52]
LC/UV-Vis	EME	NAL, DIC	24	90-98	12-500	4.0	[53]
LC/UV-Vis	DLPME	KTP, DIC, MEF	5	96-116	15.5-10,000	4.7-5.2	[51]
LC/UV-Vis	EME	KTP, NAP, DIC, IBU	10	NA	0.18-100	0.06-1.36	[54]
LC/UV-Vis	Microchip-LPME	KTP, NAP, DIC, IBU	5×10^{-3}	85-100	100 or 500-10,000	70-300	[9]
LC/UV-Vis	Semi-automatic HF-LPME	KTP, NAP, DIC, IBU	1000	90-100	0.01-1.0	0.01-0.05	[13]
CE/UV-Vis	CNF-HF-EME	NAP, IBU	4	85-88	5.0-500	1.0-1.5	[17]
LC/PD	Automatic CNF@HF-LPME coupled on-line to LC	KTP, NAP, DIC,IBU	6.4	97-105	5.0-500	1.6-4.3	This work

* Ranges listed in the Table are merely related to the target analytes in this work.

Acronyms: LC: Liquid chromatography; CE: Capillary electrophoresis, PD: Photometric detector; KTP: Ketoprofen; NAP: Naproxen; IBU: Ibuprofen; DIC: Diclofenac;

SAC: Salicylic acid; KTR: Ketorolac; NAL: Nalmefene; MEF: Mefenamic acid; NA: Not applied to real samples

4.10 Conclusion

An automatic carbon nanofiber reinforced HF-LPME coupling with HPLC method was developed for quantification of non-steroidal anti-inflammatory drugs (NSAIDs) and applied to real human urine samples. The analytes were extracted in the 3D-printed chamber with the optimal conditions, dihexyl ether as supported liquid organic solvent, 6.4 mL of sample solution. The extraction process was finished in 23 min and the sample throughput of 4 samples hr^{-1}. The analytical performance of this method provided a good linearity with the working range from 5.00 to 500.00 $\mu g \cdot L^{-1}$. The limit of detections for the method were range from 1.6-5.6 $\mu g\ L^{-1}$ and repeatability was less than 6.1%. This method is simple, automatic and environmental friendly.

CHAPTER V
CONCLUSION

5.1 Conclusion

Carbon nanomaterials reinforced hollow fiber liquid phase microextraction (HF-LPME) was developed to improve the extraction efficiency of the conventional HF-LPME. Carbon nanomaterials were added to the organic solvent membrane for additional mechanism to improve transportation of analytes. The concept has been demonstrated in the extraction of small and relatively polar organic compounds. In this research, graphite reinforced hollow fiber liquid phase microextraction was developed for extraction of trihalomethanes in water samples. The method showed that the enrichment factor or the extraction efficiency can be improved by the addition of graphite to the HF-LPME offering comparative sensitivity to the conventional HF-LPME with shorter extraction time. In addition, the in-line carbon sorbent reinforced HF-LPME was attempted and developed for fully automated analytical system. The concept has been demonstrated by coupling the carbon sorbent reinforced HF-LPME with HPLC system and developed for fully automated analysis of nonsteroidal anti-inflammatory drugs in urine samples. In this research, the extraction chamber was designed and fabricated by the 3D printer. The extraction chamber was connected and configured to the HPLC system. The method was fully automated with high sample throughput. Our researches have been proof of concept that the carbon sorbent reinforced HF-LPME can enhance extraction efficiency giving higher enrichment factor and shorter extraction time than the conventional HF-LPME, and can be coupled with an analytical system for fully automated analysis.

5.2 Suggestion for future study

This work focused on studying the reinforcement of carbon nanomaterials into the hollow fiber membrane for extraction of organic compounds. There are more nanomaterials or sorbent materials available to be explored.

In the future, this carbon nanomaterials reinforced hollow fiber membrane is probably developed and applied to electromembrane microextraction (EME) for determination of ionic species in various aqueous samples.

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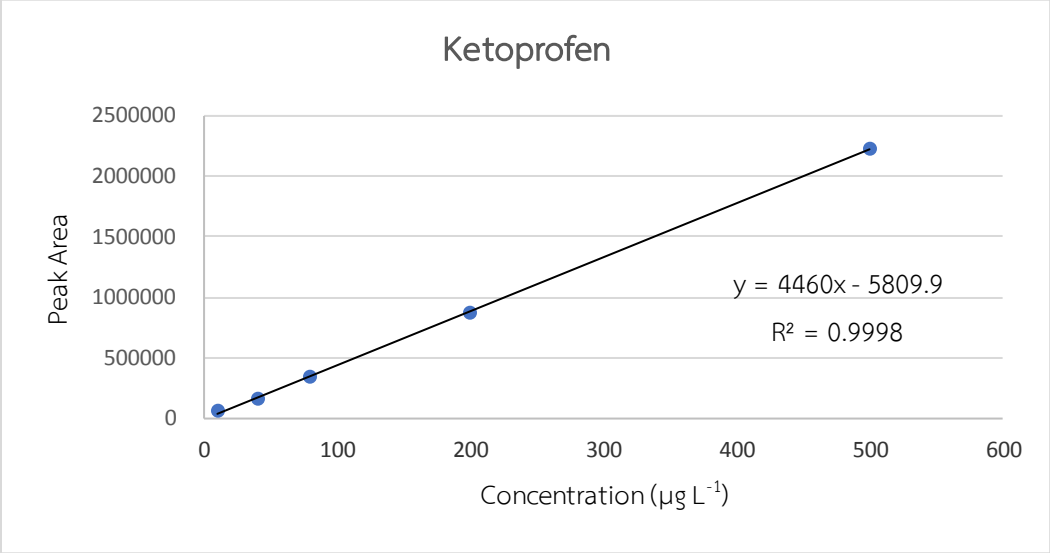
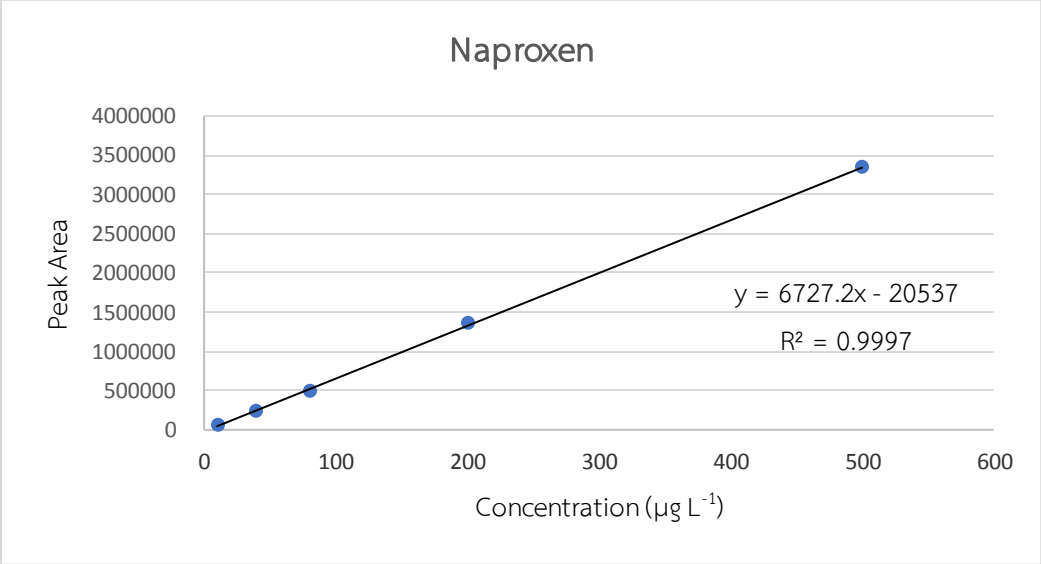
APPENDIX

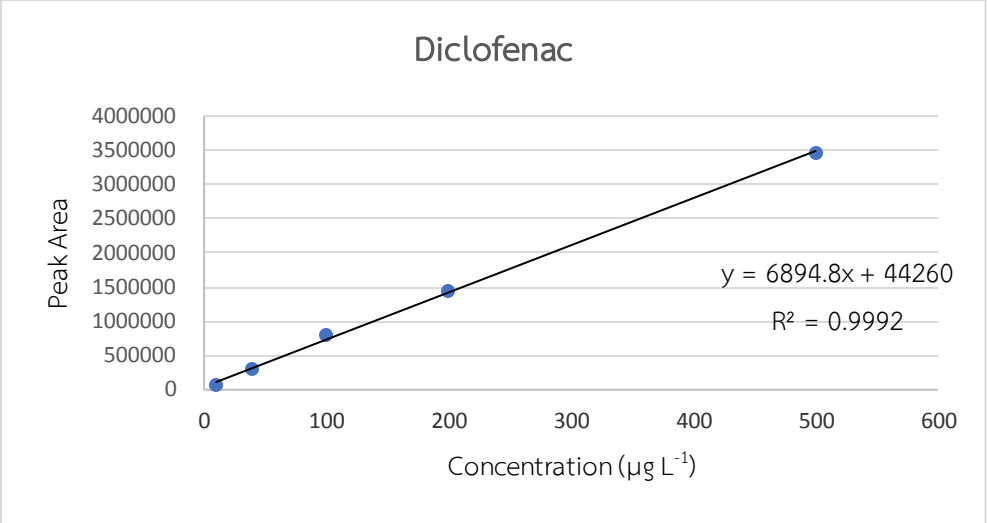
(A)


Ketoprofen
Peak Area
2500000
2000000
1500000
1000000
500000
0
0
100
200
300
400
500
600
Concentration (µg L-1)
y = 4460x - 5809.9
R² = 0.9998

(B)


Naproxen
Peak Area
4000000
3500000
3000000
2500000
2000000
1500000
1000000
500000
0
0
100
200
300
400
500
600
Concentration (µg L-1)
y = 6727.2x - 20537
R² = 0.9997

(C)



(D)

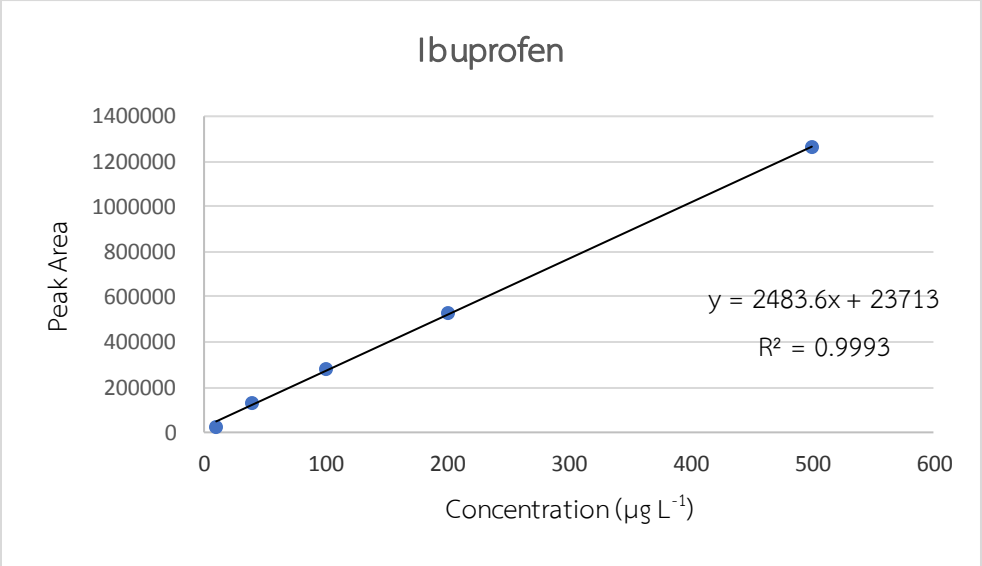


Figure A.1 Matrix-match standard calibration curve of NSAIDS determined by in-line carbon nanofiber reinforced LPME hyphenated to LC in urine. (A) ketoprofen, (B) naproxen, (C) diclofenac and (D) ibuprofen.

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